UNIVERSITY OF CALIFORNIA SAN DIEGO

Characterization of ecosystem services associated with deep-sea habitats and natural stormwater treatment systems and their incorporation into environmental management

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Oceanography with a Specialization in Interdisciplinary Environmental Research

by

Jennifer Tran Le

Committee in charge:

Professor Lisa A. Levin, Chair
Professor Eric Allen
Professor Richard T. Carson
Professor Sarah Giddings
Professor Gregory Rouse
Professor Dale Squires

2020

The Dissertation of Jennifer Tran Le is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____

_____

_____

_____

_____
Chair

University of California San Diego

2020

DEDICATION

To my family for their endless love and support.

And to everyone else who has helped along the way.

Thank you.

EPIGRAPH

"Science is magic that works."

Kurt Vonnegut, *Cat's Cradle*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

This work was only made possible by all of the people who have supported me along the way; we are almost there.

First and foremost, I would like to give the biggest thank you to Lisa Levin; I could not have asked for a better advisor. Her expertise is unrivaled, and her dedication to science is even more awe-inspiring. From day one, Lisa has pushed me to be the best scientist I can be, and she has taught me so much throughout the years. I am immensely grateful for her guidance in research, her priority in her students, and her confidence in me. She has been my most staunch supporter and my strongest advocate, encouraging of my ideas and endeavors even outside of research. I cannot imagine doing this without her.

I would also like to thank my other committee members for their excellent guidance and thoughtful advice. Thank you to the economists on my committee for making this dissertation truly interdisciplinary and stronger as a result. Dale Squires sat through countless meetings with me to work through modelling approaches and their interpretation. Our discussions always served as a reminder that science has the potential to be more than a published paper. Richard Carson has been a fount of knowledge about everything and anything. His wide range of expertise has been an invaluable resource throughout the years, helping me connect the dots in unique ways. Thank you to Gregory Rouse for being a sounding board I can always count on. He has also been incredibly supportive during at-sea expeditions, which sometimes came in the form of late-night candy bars. Thank you to Eric Allen for his thought-provoking questions that often inspired new ideas. I always came out of our meetings feeling encouraged about my research with a smile on my face. And thank you to Sarah Giddings for checking-in with me, not only as a scientist, but also as a person. She has always been in my corner, and for that I am very grateful.

The staff at SIO is absolutely phenomenal and has been immensely helpful in all aspects of my graduate school experience. Thank you to Gilbert Bretado for having all of the answers and supporting students every step of the way. His commitment to students, their well-being, and their success is truly admirable. I would also like to thank the other members of the graduate office, both past and present, for ensuring students always have what they need. Thank you to Minerva Nelson, Hanna Choe, and Adrielle Wai as well as the rest of the IOD business office for handling all of the administrative tasks necessary for SIO to function. I am extremely thankful to Penny Dockry for being the heart of CMBC and doing so much to keep it running. She has been an invaluable ally, providing endless support and resources. The PIER program has been an integral part of my graduate student experience, presenting me with countless opportunities that have helped me grow as a scientist and in my career.

Thank you to the many organizations that have provided financial and research support for my work: the Center for Marine Biodiversity & Conservation, the Stout Foundation, the UC San Diego Frontiers of Innovation Scholars Program, the Swiss Network for International Studies, USC Sea Grant, UCOP Multi-Campus Research Programs and Initiatives, the SIO Department, the UCSD Graduate Student Association, the Deep-Ocean Stewardship Initiative, Ocean Exploration Trust, Schmidt Ocean Institute, and the UC San Diego Library.

Members of the Levin Lab, past and present, have been an incredible source of knowledge and support. Lilly McCormick, Natalya Gallo, and Kirk Sato have been wonderful role models who taught me how to navigate graduate school. Olivia Pereira has been the defense-buddy I never knew I needed. Andrew Mehring, Oliver Ashford, Carlos Neira, and Guillermo Mendoza have provided invaluable research and life advice. And, essential to the Levin Lab, Jennifer Gonzalez has been a pillar of support who does so much to ensure we all keep going.

I have had the pleasure of mentoring many undergraduate students throughout the years and very much appreciate the contributions they have made to my dissertation. I would like to specifically thank Sunyoung Park for trusting me with her senior thesis; Drew Lira for loving deep-sea dive videos more than me; and Haleigh Yang for always brightening the mood with her exuberance. Additionally, I am extremely thankful to the Smith Lab for welcoming me to the field of marine science all those years ago, especially Maggie Johnson and Amanda Carter. Their continual mentorship and encouragement have irrevocably shaped my career path, and I only hope to have done the same for those I have mentored.

My friends have been an endless source of support and comfort, and I cannot imagine what graduate school would have been without them. Thank you to my Biological Oceanography cohort (Jack Pan, Alfredo Girón, and Daniel Conley) and the rest of my cohort; our shared struggles have become our shared triumphs throughout the years. Thank you to Neosho (Bethany Kolody, Beverly French, Kiefer Forsch, Sam Honch, Kaitlyn Lowder, and James Giammona) for providing this gremlin with a home full of friendship. Thank you to Angela Zoumplis and Sarah Schwenk for reminding me to have fun during graduate school; to Canon Purdy, Michele Heller, and Lauren Ward for showing me that there is more to science than research; and to Oriana Poindexter and Chase Martin for making sure I get my daily dose of sunlight. I would also like to thank Alix Ramsey, Denise Francesca, and Rae Collins for believing in me when I could not; Jessica Aceret and April Flores for understanding me on a deeper level; Shelley Kuang and Amy Le for inspiring me to be my best-self; Sloan Turner, Jaclynn Do, and Sarah Stanford for being there since the beginning; and BTS for being my bulletproof shield.

And finally, I am eternally thankful for my family. My bby seastar Brittney has always been my most trusted confidant, my number one fan, and my best friend. My parents are the

most loving and supportive people in my life, providing me with everything I needed in order to succeed. I greatly appreciate all they have sacrificed for me throughout my life. Their love knows no bounds and is the source of my drive and my ambition. They are my biggest motivation and I only hope to have made them proud.

Chapter 2, in full, is a reprint of the material as it appears in Le, J.T., Levin, L.A., and R.T. Carson. 2017. Incorporating ecosystem services into environmental management of deep-seabed mining. Deep-Sea Research II. 137, 486-503. DOI: 10.1016/j.dsr2.2016.08.007. The dissertation author was the primary investigator and author of this material. The material is used by permission of Elsevier.

Chapter 3, in part, is in preparation for submission for publication. Le, J.T., Levin, L.A., Lejzerowicz, F., Cordier, T., Gooday, A., and J. Pawlowski. Scientific and economic tradeoffs between morphological and molecular methods for biodiversity assessment in the deep sea. The dissertation author was the primary investigator and author of this material.

Chapter 4, in part, is in preparation for submission for publication. Le, J.T., Girguis, P., and L.A. Levin. Using deep-sea imagery to examine ecosystem services associated with methane seeps. The dissertation author was the primary investigator and author of this material.

Chapter 5, in part, is in preparation for submission for publication. Le, J.T., Gonzalez, J., Carson, R.T., Ambrose, R.F. and L.A. Levin. Ecosystem services associated with natural stormwater treatment systems in Los Angeles: A review. The dissertation author was the primary investigator and author of this material.

Chapter 6, in part, is in preparation for submission for publication. Le, J.T., Mehring, A.S., Gonzalez, J.P., Park, S., Kurylo, J., and L.A. Levin. Carbon efficiency of managed urban

landscapes in southern California. The dissertation author was the primary investigator and author of this material.

VITA

2014	Bachelor of Science, Environmental Systems (Ecology, Behavior, and Evolution), University of California San Diego
2014	Bachelor of Arts, Honors of Distinction, Economics, University of California San Diego
2017	Master of Science, Oceanography, Scripps Institution of Oceanography, University of California San Diego
2020	Doctor of Philosophy, Oceanography with a Specialization in Interdisciplinary Environmental Research, Scripps Institution of Oceanography, University of California San Diego

PUBLICATIONS

Le, J.T., Levin, L.A., and R.T. Carson. 2017. Incorporating ecosystem services into environmental management of deep seabed mining. Deep-Sea Research II. 137: 486-503. DOI: 10.1016/j.dsr2.2016.08.007.

ABSTRACT OF THE DISSERTATION

Characterization of ecosystem services associated with deep-sea habitats and natural stormwater treatment systems and their incorporation into environmental management

by

Jennifer Tran Le

Doctor of Philosophy in Oceanography with a Specialization in Interdisciplinary Environmental Research

University of California San Diego, 2020

Professor Lisa A. Levin, Chair

We, as a society, are notoriously bad at finding balance between extraction of natural resources and environmental protection. The concept of ecosystem services, the direct and indirect benefits derived from the environment, attempts to ameliorate these failures by linking natural processes to human well-being. The goal of this dissertation was to explore approaches for characterizing ecosystem services and to identify how they can be incorporated into

environmental management. To do this, I used two groups of systems subject to human impact, deep-sea habitats and natural stormwater treatment systems (NTS), that provided a suite of characteristics with which to compare and contrast (e.g. marine versus terrestrial, level of human impact, ease of access). While deep-sea habitats and NTS provide some of the same ecosystem services, the structures and functions that support them can differ. Mechanisms for incorporating this information into environmental decision-making differ among systems as well. As interest in deep-sea natural resources continues to grow, environmental decision-makers have the novel opportunity to employ an ecosystem services approach, prior to commercial exploitation in cases such as mining. In comparing molecular and morphology-based methods for assessment and monitoring of deep-sea biodiversity, I examined scientific and economic tradeoffs between the two to suggest a combined approach as most cost-effective when considering future environmental requirements. I also leveraged existing deep-sea imagery and biological trait analysis to evaluate fisheries services and climate-regulating services related to carbon at methane seeps off southern California, identifying the Del Mar seep as the largest contributor to ecosystem services. In contrast to the seemingly untouched deep sea, NTS are human-designed to mimic physical and biological processes, which can generate ecosystem services such as climate-regulating services related to carbon. I found that, although urban greenspaces are not carbon sinks, NTS and natural areas are more carbon-efficient than grass lawns and horticultural gardens. NTS present a unique opportunity to manipulate natural structures and functions for targeted benefits, such as carbon sequestration and storage. Together, this body of work serves to operationalize ecosystem services in a multitude of contexts with practical applications.

Chapter: 1 Introduction

Jennifer T. Le

As human impact increases in marine and coastal ecosystems (Halpern et al., 2015), rules, regulations, and policies have struggled to keep up with the need for adequate environmental management (Mengerink et al., 2014). There has long been an emphasis on biodiversity conservation in environmental decision-making (e.g. United Nations Convention on Biological Diversity), but biodiversity for biodiversity's sake is not always enough in the face of industry and economic development. However, biodiversity is often considered the source of most ecosystem services (Palumbi et al., 2009; Science for Environment Policy, 2015), which are the direct and indirect benefits derived from the environment.

The concept of ecosystem services was made popular by the Millennium Ecosystem Assessment (MEA, 2005), and has since gone through many iterations (e.g. IPBES, 2013; Haines-Young & Potschin, 2018). I adopt the following framework: ecosystems have physical, chemical, and biological *structures* that support ecological *functions* (or supporting services), which can be combined with a human perspective to produce ecosystem *services*. They are divided into four categories: provisioning (goods and products, e.g. fisheries landings), regulating (environmental regulating processes, e.g. carbon sequestration and storage), cultural (non-material benefits, e.g. recreation and aesthetic value), and supporting (ecological functions that support final services, e.g. primary production and essential fish habitat). Ecosystem services provide a direct link between natural systems and human well-being, contributing to a tangible

rationale for sustainable development, management, and protection of the environment. As a result, they can be a useful tool in environmental decision-making.

This dissertation aimed to explore approaches for characterizing ecosystem services in two types of systems subject to human impact: deep-sea habitats (Chapters 2-4) and natural stormwater treatment systems (Chapters 5-6). The broad scope of systems highlights the context-dependence of ecosystem services as well as overarching themes among them. I employed an interdisciplinary approach that was heavily influenced by the ecology and economic disciplines, which facilitated application of results to real questions in environmental management.

In the deep sea, there is increasing demand for natural resources (Merrie et al., 2014), but environmental rules, regulations, and policies are only now being developed for some parts of it (e.g. ISA, 2019). The emerging industry of deep-seabed mining presents a novel opportunity to incorporate ecosystem services into environmental management prior to commercial exploitation. Chapter 2 reviews ecosystem services associated with deep-sea habitats targeted for mining (continental margins, seamounts, hydrothermal vents, and abyssal plains), and the structures and functions that support them. Known examples include fisheries landings (Victorero et al., 2018), genetic resources (Harden-Davies, 2017), carbon sequestration and storage (Sweetman et al., 2019), and element cycling (Blöthe et al., 2015), but there are likely to be ecosystem services yet discovered. Chapter 2 also discusses how and where ecosystem services can be incorporated into existing deep-seabed mining regulations. Overall, the second chapter of this dissertation creates a framework for studying ecosystem services and for applying them to environmental decision-making.

Chapter 3 examines one piece of environmental management that needs to be addressed for deep-seabed mining: biodiversity assessment and monitoring. Multiple approaches are

available to conduct biodiversity assessment and monitoring, such as morphology-based taxonomy and metabarcoding (Lejzerowicz et al., 2015). The former involves an expert hand-picking organisms out of an environmental sample and identifying them by eye or with the aid of a microscope. The latter involves chemical processing, molecular sequencing, and bioinformatics. Each of these methods are associated with scientific advantages and disadvantages, including how well they inform ecosystem services, and specific economic costs. In addition to discussing these scientific tradeoffs, Chapter 3 compares their cost-effectiveness in identifying deep-sea organisms, which is relevant to decision-makers when considering assessment and monitoring requirements.

Another approach often employed in deep-sea research is the use of remotely-operated vehicles (ROVs) to survey and collect samples, including a wealth of imagery data. In the summer of 2015, Ocean Exploration Trust completed an expedition to explore the southern California borderlands, which included ROV dives at several methane seeps on the continental margin (Levin, 2016a). Methane seeps are habitats where hydrocarbons and other fluids escape from the seafloor, fueling a biological community based on chemosynthesis (Orphan et al., 2001; Levin, 2005). In a widely food-limited environment, elevated primary production could significantly influence adjacent areas (Levin et al., 2016b). Chapter 4 uses ROV dive videos from three methane seeps to demonstrate how deep-sea imagery can be used for characterization of ecosystem services. More specifically, Chapter 4 focuses on fisheries services and climate-regulating services related to carbon, which have previously been documented at a California methane seep (Grupe et al., 2015). It provides the first detailed biological description of two methane seeps, and is the first application of an ecosystem services-based trait approach in the deep sea.

Ecosystem services can also be generated by built ecosystems, such as natural stormwater treatment systems (NTS). NTS are human-made installations that are designed to capture and treat stormwater runoff using physical and biological processes (Payne et al., 2015). Coastal development and urbanization have altered water flows and introduced contaminants into runoff, which can cause flooding of infrastructure and pollution of local water (Walsh et al., 2005). NTS provide a low-impact strategy to address these issues by slowing water flows, removing contaminants, and storing runoff for possible reuse (Hatt et al., 2009; Askarizadeh et al., 2015). In southern California, where there is a discrepancy between water supply and demand, NTS are becoming more widespread (Ambrose & Winfrey, 2015). Chapter 5 reviews potential ecosystem services associated with NTS, such as targeted water infiltration and pollutant removal as well as non-targeted pollination, climate regulation, aesthetic value, and pests (disservice). Examples from Los Angeles County illustrate these ecosystem services and practical methods are suggested to begin quantifying and economically valuating them.

Chapter 6 investigates one ecosystem service associated with NTS: climate regulation related to carbon. Vegetation within NTS, such as biofilters and bioswales, uptake atmospheric carbon dioxide through photosynthesis and can store it as biomass or in soil. Plant and soil respiration, the reverse process that releases carbon back into the atmosphere, is dependent on environmental characteristics such as soil moisture and soil temperature (Carey et al., 2016). Chapter 6 provides measurements of carbon fluxes over NTS, in comparison to other urban land uses in San Diego (USA): grass lawns, horticultural gardens, and natural coastal sage scrub. Data envelopment analysis is used to compare carbon efficiency, i.e. how well each system converts its given environmental characteristics into desirable carbon fluxes. This analysis incorporates

both environmental inputs and outputs, which may be helpful when considering urban management strategies.

The final chapter of this dissertation summarizes the lessons learned from applying an ecosystem services perspective in multiple systems using multiple approaches. It also provides recommendations for incorporating ecosystem services into environmental decision-making and management.

References

Ambrose, R. F., and B. K. Winfrey. 2015. Comparison of stormwater biofiltration systems in Southeast Australia and Southern California. WIREs Water 2:131–146.

Askarizadeh, A., M. A. Rippy, T. D. Fletcher, D. L. Feldman, J. Peng, P. Bowler, A. S. Mehring, B. K. Winfrey, J. A. Vrugt, A. Aghakouchak, S. C. Jiang, B. F. Sanders, L. A. Levin, S. Taylor, and S. B. Grant. 2015. From Rain Tanks to Catchments: Use of Low-Impact Development To Address Hydrologic Symptoms of the Urban Stream Syndrome. Environmental Science and Technology 49:11264–11280.

Blöthe, M., A. Wegorzewski, C. Müller, F. Simon, T. Kuhn, and A. Schippers. 2015. Manganese-Cycling Microbial Communities Inside Deep-Sea Manganese Nodules. Environmental Science and Technology 49:7692–7700.

Carey, J. C., J. Tang, P. H. Templer, K. D. Kroeger, T. W. Crowther, A. J. Burton, J. S. Dukes, B. Emmett, S. D. Frey, M. A. Heskel, L. Jiang, M. B. Machmuller, J. Mohan, A. M. Panetta, P. B. Reich, S. Reinschj, X. Wang, S. D. Allison, C. Bamminger, S. Bridgham, S. L. Collins, G. De Dato, W. C. Eddy, B. J. Enquist, M. Estiarte, J. Harte, A. Henderson, B. R. Johnson, K. S. Larsen, Y. Luo, S. Marhan, J. M. Melillo, J. Peñuelas, L. Pfeifer-Meister, C. Poll, E. Rastetter, A. B. Reinmann, L. L. Reynolds, I. K. Schmidt, G. R. Shaver, A. L. Strong, V. Suseela, and A. Tietema. 2016. Temperature response of soil respiration largely unaltered with experimental warming. Proceedings of the National Academy of Sciences of the United States of America 113:13797–13802.

Convention on Biological Diversity. 2008. IX/20. Marine and coastal biodiversity UNEP/CBD/C.

Grupe, B. M., M. L. Krach, A. L. Pasulka, J. M. Maloney, L. A. Levin, and C. A. Frieder. 2015. Methane seep ecosystem functions and services from a recently discovered southern California seep. Marine Ecology 36:91–108.

Haines-Young, R., and M. Potschin. 2018. CICES V5. 1. Guidance on the Application of the Revised Structure.

Halpern, B. S., M. Frazier, J. Potapenko, K. S. Casey, K. Koenig, C. Longo, J. S. Lowndes, R. C. Rockwood, E. R. Selig, K. A. Selkoe, and S. Walbridge. 2015. Spatial and temporal changes in cumulative human impacts on the world's ocean. Nature Communications 6.

Harden-Davies, H. 2017. Deep-sea genetic resources: New frontiers for science and stewardship in areas beyond national jurisdiction. Deep-Sea Research Part II: Topical Studies in Oceanography 137:504–513.

Hatt, B. E., T. D. Fletcher, and A. Deletic. 2009. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. Journal of Hydrology 365:310–321.

International Seabed Authority. 2019. Draft regulations on exploitation of mineral resources in the Area. ISBA/25/C/WP.1. Kingston, Jamaica.

IPBES. 2014. Conceptual framework for the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services IPBES-2/4.

Lejzerowicz, F., P. Esling, L. Pillet, T. A. Wilding, K. D. Black, and J. Pawlowski. 2015. High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. Scientific Reports 5.

Levin, L. A. 2005. Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology 43:1–46.

Levin, L., P. Girguis, C. German, M. Brennan, S. Tüzün, J. Wagner, C. Smart, A. Kruger, K. Inderbitzen, J. Le, M. Martinez, C. Martinez, E. Kappel, N. Gallo, and B. Grupe. 2016a. Exploration and discovery of methane seeps and associated communities in the California Borderland. New Frontiers in Ocean Exploration: The E/V Nautilus and NOAA Ship Okeanos Explorer 2015 Field Season 29:40–43.

Levin, L. A., A. R. Baco, D. A. Bowden, A. Colaco, E. E. Cordes, M. R. Cunha, A. W. J. Demopoulos, J. Gobin, B. M. Grupe, J. Le, A. Metaxas, A. N. Netburn, G. W. Rouse, A. R. Thurber, V. Tunnicliffe, C. L. Van Dover, A. Vanreusel, and L. Watling. 2016b. Hydrothermal vents and methane seeps: Rethinking the sphere of influence. Frontiers in Marine Science 3.

Mengerink, K. J., C. L. Van Dover, J. Ardron, M. Baker, E. Escobar-briones, K. Gjerde, J. A. Koslow, E. Ramirez-llodra, A. Lara-lopez, D. Squires, T. Sutton, A. K. Sweetman, and L. A. Levin. 2014. A Call for Deep-Ocean Stewardship. Science 344:696–698.

Merrie, A., D. C. Dunn, M. Metian, A. M. Boustany, Y. Takei, A. O. Elferink, Y. Ota, V. Christensen, P. N. Halpin, and H. Österblom. 2014. An ocean of surprises - trends in

human use, unexpected dynamics and governance challenges in areas beyond national jurisdiction. Global Environmental Change 27:19–31.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington DC.

Orphan, V. J., K. Hinrichs, W. Ussler, C. K. Paull, L. T. Taylor, S. P. Sylva, J. M. Hayes, and E. F. Delong. 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Applied and Environmental Microbiology 67:1922–1934.

Palumbi, S. R., P. A. Sandifer, J. D. Allan, M. W. Beck, D. G. Fautin, M. J. Fogarty, B. S. Halpera, L. S. Incze, J. A. Leong, E. Norse, J. J. Stachowicz, and D. H. Wall. 2009. Managing for ocean biodiversity to sustain marine ecosystem services. Frontiers in Ecology and the Environment 7:204–211.

Payne, E., B. Hatt, A. Deletic, M. Dobbie, D. McCarthy, and G. Charasena. 2015. Adoption Guidelines for Stormwater Biofiltration Systems - Summary Report. Melbourne.

Science for Environment Policy. 2015. Ecosystem Services and Biodiversity.

Sweetman, A. K., C. R. Smith, C. N. Shulse, B. Maillot, M. Lindh, M. J. Church, K. S. Meyer, D. van Oevelen, T. Stratmann, and A. J. Gooday. 2019. Key role of bacteria in the short-term cycling of carbon at the abyssal seafloor in a low particulate organic carbon flux region of the eastern Pacific Ocean. Limnology and Oceanography 64:694–713.

Victorero, L., L. Watling, M. L. D. Palomares, and C. Nouvian. 2018. Out of sight, but within reach: A global history of bottom-trawled deep-sea fisheries from > 400 m depth. Frontiers in Marine Science 5.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan II. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of North American Benthological Society 24:706–723.

Chapter 2: Incorporating ecosystem services into environmental management of deep-seabed mining

This chapter is presented as a paper. “Incorporating ecosystem services into environmental management of deep-seabed mining” was published in *Deep-Sea Research Part II: Topic Studies in Oceanography* in 2017.

Deep-Sea Research II 137 (2017) 486–503

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Regular article

Incorporating ecosystem services into environmental management of deep-seabed mining

Jennifer T. Le [a,*], Lisa A. Levin [a], Richard T. Carson [b]

[a] Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, La Jolla, CA 92093-0218, United States
[b] Department of Economics, University of California, San Diego, La Jolla, CA 92093-0508, United States

ARTICLE INFO

Available online 21 August 2016

Keywords:
Deep-seabed mining
Benthic environment
Benthic communities
Ecosystem management
Environmental impact
International policy

ABSTRACT

Accelerated exploration of minerals in the deep sea over the past decade has raised the likelihood that commercial mining of the deep seabed will commence in the near future. Environmental concerns create a growing urgency for development of environmental regulations under commercial exploitation. Here, we consider an ecosystem services approach to the environmental policy and management of deep-sea mineral resources. Ecosystem services link the environment and human well-being, and can help improve sustainability and stewardship of the deep sea by providing a quantitative basis for decision-making. This paper briefly reviews ecosystem services provided by habitats targeted for deep-seabed mining (hydrothermal vents, seamounts, nodule provinces, and phosphate-rich margins), and presents practical steps to incorporate ecosystem services into deep-seabed mining regulation. The linkages and translation between ecosystem structure, ecological function (including supporting services), and ecosystem services are highlighted as generating human benefits. We consider criteria for identifying which ecosystem services are vulnerable to potential mining impacts, the role of ecological functions in providing ecosystem services, development of ecosystem service indicators, valuation of ecosystem services, and implementation of ecosystem services concepts. The first three steps put ecosystem services into a deep-seabed mining context; the last two steps help to incorporate ecosystem services into a management and decision-making framework. Phases of environmental planning discussed in the context of ecosystem services include conducting strategic environmental assessments, collecting baseline data, monitoring, establishing marine protected areas, assessing cumulative impacts, identifying thresholds and triggers, and creating an environmental damage compensation regime. We also identify knowledge gaps that need to be addressed in order to operationalize ecosystem services concepts in deep-seabed mining regulation and propose potential tools to fill them.

1. Introduction

The deep sea contains many highly heterogeneous ecosystems that host a vast, but not yet fully quantified wealth of biological, energy, and mineral resources (Ramirez-Llodra et al., 2010; Mengerink et al., 2014). Benefits from these natural resources include food, fuel, raw materials, and non-market benefits (Thurber et al., 2014). As industries begin to use deep-sea resources in order to meet growing demand for food, pharmaceuticals, energy, and minerals, how these benefits are produced and maintained grows increasingly important to understand. However, many knowledge gaps still exist regarding how ecosystem structure and ecological functions translate into benefits to society. Parsing through these relationships is essential to the long-term, sustainable, and effective environmental policy and management of deep-sea ecosystems subject to exploitation.

For much of the past century, deep-sea research has focused on biological community structure by defining abundance, distribution, and diversity (Rex and Etter, 2010). More recently, there has been a shift in emphasis towards how structure, biodiversity in particular, supports ecological functions (Danovaro et al., 2008, 2016; Thurber et al., 2014). Biodiversity is often heralded as necessary to provide most ecosystem services (ES), i.e. the contributions to human well-being from ecosystems, and is used as a proxy for measuring these services (Palumbi et al, 2009; Cardinale et al., 2012). In this paper, biodiversity will be discussed as a component of ecosystem structure because it has been shown to contribute to ecological function and ES capacity (Worm et al., 2006; Harrison et al., 2014; Yasuhara et al., 2016). The relationship between biodiversity and ES remains unclear in many cases (Balvanera et al., 2014; Bennett et al., 2015), perhaps even more so in the deep sea where biodiversity is not yet well characterized

* Corresponding author.
E-mail address: jtl025@ucsd.edu (J.T. Le).

http://dx.doi.org/10.1016/j.dsr2.2016.08.007
0967-0645/

(Higgs and Attrill, 2015; Sinniger et al., 2016). However, one of the largest anticipated deep-seabed mining (DSM) impacts is loss of biodiversity and its contribution to ES should not be ignored.

Many of the ecological functions that ecosystem structure supports can ultimately be translated into ES. For example, seamount-trapped, vertically-migrating zooplankton (structure) can provide trophic support (function) for fish catch (service) (Clark et al., 2010). Another example is deep-sea infauna (structure) that facilitate the burial of carbon in deep sediments via bioturbation (function), which contributes to carbon sequestration and climate regulation (service) (Xiao et al., 2010). The publication of the Millennium Ecosystem Assessment (MA) (2005) stimulated interest in examining ES and developing ES frameworks for environmental decision-making (Fisher et al., 2009). ES try to associate values with environmental benefits that are linked to human well-being, whether a market exists for the benefit or not. Sustainable management of resources requires that these values are incorporated into environmental regulation.

Deep-sea exploration began in the 1800s but exploitation of its natural resources is a more recent development. There is a growing list of anthropogenic impacts in the deep sea (Ramirez-Llodra et al., 2011) which can result in the loss of ES, including ES yet to be discovered. Fisheries are encroaching deeper into the water column and on the seabed (Morato et al., 2006; Watson and Morato, 2013). The overexploitation of fisheries species by direct targeting or removal as bycatch may cause deep-sea fish populations to decline precipitously. Population declines and crashes may have longer-lasting effects in the deep sea relative to shallow water because life spans are much longer at great depths (Devine et al., 2006; Norse et al. 2012). In addition, trawl fisheries cause physical disturbance and removal of habitat, leaving coral rubble and trawl marks (Roberts, 2002; Puig et al., 2012; Buhl-Mortensen et al., 2015). The removal of three-dimensional habitat structure on the bottom causes loss of associated species that are very slow or unable to recover (Althaus et al., 2009; Williams et al., 2010). Trawling also alters sediment flux and re-suspends sediment in the water column, which can lead to lower biodiversity and ecological function (Martín et al., 2014; Pusceddu et al., 2014; Oberle et al., 2016).

Oil and gas exploration and drilling are now taking place in increasingly deeper waters (Merrie et al., 2014). The infrastructure and extraction of these energy resources have direct impacts on the deep seafloor (Continental Shelf Associates, Inc., 2006). With deeper oil comes an increasing risk of oil spills (e.g. *Deepwater Horizon*, Reddy et al., 2012; Merrie et al., 2014), which have the potential to result in both the loss of deep-sea habitats (White et al., 2012; Fisher et al., 2014), as well as losses of ES in shallow water and coastal systems (Lin and Mendelssohn, 2012).

With accelerating exploration claims in both national and international deep waters, DSM is expected to commence in the near future. Since the first exploration contracts were signed in 2001 (Lévy, 2014), the International Seabed Authority (ISA) has approved 27 contracts in the Pacific, Atlantic, and Indian oceans for polymetallic sulfides, ferromanganese crusts, and polymetallic nodules. Eighteen of these contracts were granted within the last five years (Wedding et al., 2015). The ISA was established by the United Nations Convention on the Law of the Sea (UNCLOS) and governs the minerals and environment in the "Area," defined as the seabed beyond national jurisdiction (UNCLOS, 1982).

Regulation exists for the exploration of polymetallic sulfides, ferromanganese crusts, and polymetallic nodules, but it is not yet in place to ensure the protection of the environment under commercial exploitation (ISA, 2015, 2016). The ISA has made recommendations regarding baseline data collection and monitoring plans (ISA, 2013a), but environmental regulation is still under development. Because commercial DSM has yet to begin, there is an opportunity to incorporate ES indicators into data-collection requirements in all phases of environmental management and decision-making. An ES framework can provide guidance on how valuable services might be maintained while still yielding benefits from the direct extraction of natural resources.

The objectives of this paper are to (1) review ES associated with deep-sea mineral resources and their host habitats; (2) propose practical steps to build ES into environmental planning of DSM; this includes the identification of potentially vulnerable ES, the role of ecosystem structure and ecological function in providing ES, their use as ES indicators, and the valuation of ES; (3) indicate management phases where ES could be incorporated; and (4) identify scientific knowledge gaps that must be addressed to implement an ES framework for DSM regulation.

2. Application of an ecosystem services approach to the deep sea

ES are the contributions to human well-being from ecosystems. MA (2005) categorizes ES into four groups: provisioning, regulating, cultural, and supporting. Provisioning services are the outputs and products obtained from ecosystems; examples include fish and invertebrate catch, pharmaceuticals, and industrial agents (MA, 2005). There is some controversy over the inclusion of abiotic resources as provisioning services because their formation does not involve biotic processes and the timescale associated with their formation is extremely long. Our focus here is on the role of biotic ES in decision-making and planning, partly to identify areas where biotic ES losses can be minimized while still allowing extraction of abiotic resources. Regulating services are benefits from the regulation of environmental processes (MA, 2005). A deep-sea example would be promoting carbon sequestration through transport of carbon to the seabed for burial via the biological pump and diurnal vertical migrations. Another example includes biological regulation, which here will refer to the biological control of populations and pests (Armstrong et al., 2012). Cultural services are non-material benefits that include educational opportunities, aesthetic considerations (e.g. inspiration for the arts), the utility obtained simply from knowing the resource exists, and that the public is being a good steward of the resource for both the current and future generations. The underlying motive for valuing ES is, in many instances, maintaining the option to use these ES at some point in the future. The concept of quasi-option value, where investments are made in scientific research to improve knowledge of the ES, is particularly relevant because knowledge concerning deep-sea ES is often quite limited (Carson et al., 1999). When extractive activities pose the threat of irreversible harm, this consideration can be particularly large. The MA also defines supporting services as those necessary for the production of all other ES, which includes primary and secondary production, and element and nutrient cycling (MA, 2005).

A number of alternative classification systems for ES exist (e.g. Böhnke-Henrichs, et al., 2013; Landers and Nahlik, 2013; Liquete et al., 2013). Two that are commonly used are The Economics of Ecosystems and Biodiversity (TEEB) and the Common International Classification of Ecosystem Services (CICES). TEEB defines function as a subset of ecological processes with the potential or capacity to provide a service. Services are then defined as the realization of the function that provides a benefit to human well-being (de Groot et al., 2010). CICES defines final ES as contributions to human well-being while ecosystem goods and benefits are created or derived from final ES (Haines-Young and Potschin, 2013). Unlike the MA, both TEEB and CICES exclude supporting services from their classification, although both systems acknowledge their importance. What TEEB and CICES define as

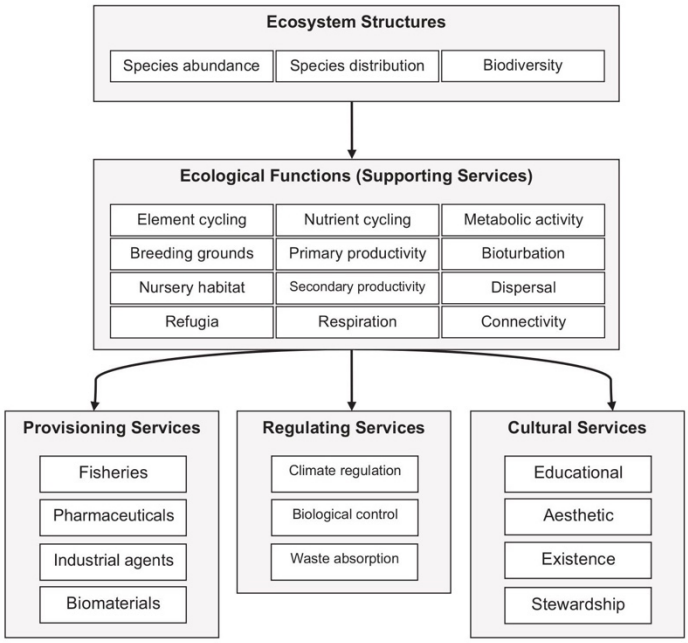

Fig. 1. An overview of linkages between biological ecosystem structures, ecological functions (supporting services), and the ecosystem services (provisioning, regulating, and cultural) they support.

"function" is similar to the MA category of "supporting service," as both are characterized as ecological processes that contribute to ES capacity. It has been argued that the value of supporting services is included in the value of the final services to which they contribute and including them separately would result in double-counting (Boyd and Banzhaf, 2007; Fisher et al., 2009) and an overestimate of economic value. Both the TEEB and CICES systems include instead a distinction between service and benefit to avoid double-counting and to acknowledge that multiple benefits can be derived from one service (e.g. fish and invertebrate catch can provide both food and livelihoods). However, this paper will consider services and benefits together for simplification.

For this discussion we will use a modified form of the TEEB classification system (Fig. 1). ES are the direct and indirect contributions to human well-being, which are grouped into three categories: provisioning, regulating, and cultural. The TEEB considers habitat, including life-cycle maintenance and gene pool protection, its own category of ES but this paper will consider elements within this category as functions (or supporting services as defined by the MA), i.e. ecological processes with the potential or capacity to provide a service. ES are the results of ecological functions that are supported by ecosystem structure defined as the physical, chemical, and biological characteristics of a system. For example, the corals and sponges on seamounts acts as habitat and aggregate fish and their prey (structure), generating trophic interactions and secondary production (functions). These interactions result in fish catch (service), leading to economic and social welfare in the form of food provision and livelihoods. If structure and function are not explicitly identified and protected, then the service may not continue. Ecological functions (supporting services) may be of elevated importance in the context of DSM. Their inclusion in economic valuation can increase estimates of the benefits of alternative development options that are less disruptive. It is essential to highlight their contribution to final ES in order to correctly assess the value of protecting them.

DSM impacts could potentially affect these components of ES provision directly or the linkages among them. Linkages among structure, function, and service must be understood to predict how DSM will affect the provision of ES. Understanding the translation between structure and function and between function and service is essential in order to develop optimal ES indicators, calculate the value of environmental damage, and provide a more complete knowledge of deep-sea processes.

An ES approach has been previously applied to conservation of terrestrial and shallow-water systems, including forests (e.g. Chazdon, 2008; Seidl et al., 2016), coral reefs (e.g. Farber et al., 2002; Rogers et al., 2015), and wetlands (e.g. Aburto-Oropeza et al., 2008; Gunderson et al., 2016) among many other examples. These ecosystems have been and are still subject to destructive practices, including deforestation, coral dynamiting, and conversion to shrimp farms. Incorporation of an ES perspective into environmental decision-making can initiate re-evaluation of these practices. For example, the deforestation of coastal mangroves in the Gulf of California destroys nursery habitat for commercially-important fish species, resulting in loss of profit for local fisheries (Aburto-Oropeza et al., 2008). Despite its integral role in supporting a profitable fishery, the ES of nursery habitat by mangroves was previously ignored.

Linkages between shallow-water ecosystems and human well-being are much better defined than the linkages between deep-sea ecosystems and human well-being. Wetland habitats may provide some similar services as the deep sea such as genetic resources and carbon sequestration (Chmura et al., 2003). However, because they are in closer proximity to human establishments, wetland habitats also provide more direct services, such as coastal storm and surge buffering, shoreline stabilization, and flood prevention

(Koch et al., 2009; Barbier et al., 2011; Gedan et al., 2011), in addition to waste absorption and climate regulation, which are also provided by the deep sea (Armstrong et al., 2012; Thurber et al., 2014). These well-defined services have helped support wetland conservation, such as the U.S. no net wetland loss policy (U.S. Fish and Wildlife, 2002). The conservation value of wetlands will be seen as increasingly important as wetland climate mitigation potential is recognized (Mcleod et al., 2011; Hopkinson et al., 2012). Whether this holds true for the deep sea remains to be seen (Levin and Le Bris, 2015).

Deep-sea ES differ from terrestrial and shallow water ES because the structures and functions (supporting services) that support them, and consequently the ES they provide, are thought to be largely non-restorable. The restoration of DSM sites will be extremely costly with questionable success because of the inaccessibility of the deep sea and lack of knowledge regarding how it functions (Van Dover et al., 2014a). Deep-sea ES are distinct from many other marine and terrestrial systems because (a) there is a large spatial separation between where the service is provided and the stakeholders benefitting from it; (b) many deep-sea processes operate on extremely long time scales (McMurtry, 2001; Devine et al., 2006); and (c) there are significant unexplored and undiscovered constituents and processes in many deep-sea habitats (Ramirez-Llodra et al., 2010). These unknowns can have potentially large-scale consequences if the extraction of deep-sea minerals results in the loss of an undiscovered ES integral to human well-being. A better understanding of the deep sea must be established in order to preserve both use and non-use value provided by its many habitats and species. Because commercial mining has not yet started, the ISA has the opportunity to implement a system for evaluating ES impacts from the start rather than after serious problems arise, as has typically been the case with other ecosystems (e.g. terrestrial forests).

The concept of ES has not been widely applied to deep-sea resource management. Both Boschen et al. (2013) and Collins et al. (2013) address environmental management for polymetallic sulfide mining at hydrothermal vents, but do not mention ES in their recommendations. Van Dover et al. (2014a) do include compensation for harm to ES as a potential source of funding for deep-sea restoration.

One of the few examples of the application of ES to deep-sea resources comes from Batker and Schmidt (2015), who use terrestrial mining metrics as a template for assessing DSM impacts at the Solwara I hydrothermal vent, a polymetallic sulfide site in Papua New Guinea. This report was commissioned by Nautilus Minerals as a preliminary examination of ES that may be impacted by the Solwara I project. The authors conclude that DSM is necessary to meet global demand for copper and will impact ES to a lesser extent relative to terrestrial mines. The use of terrestrial mining metrics for comparison in this report has drawn criticism from Rosenbaum and Grey (2015, http://www.deepseaminingoutofourdepth.org/wp-content/uploads/accountabilityZERO_web.pdf). Some of the terrestrial ES used in the assessment, such as water supply and soil formation, are not relevant to the deep sea. Unique deep-sea ES, like the cycling of sulfur and iron (Tagliabue et al., 2010; Resing et al., 2015) or industrial agents (Mahon et al., 2015), are overlooked. Deep-sea ES that have not been discovered but potentially exist (based on findings in other reducing ecosystems), such as novel nursery grounds (Levin et al., 2016), food provision, and pharmaceuticals, were assumed to have no economic value. Although the Solwara I project is one site, it is important to consider its impacts in conjunction with the possibility of additional deep-sea activities in the region which are actively being planned, e.g. the Solwara 12 project by Nautilus Minerals (Golder Associates, 2012) as well as their exploration work in Tonga and the Solomon Islands and deep-sea mine tailings placement in Papua New Guinea (Shimmield et al., 2010). There are also potential inconsistencies within the report. For example, Batker and Schmidt (2015) state that hydrothermal vents are unique systems with endemic species but then later say that the DSM impact on genetic resources will be low. The assessment treats Solwara I as an isolated system and does not examine its larger role in the deep sea via connectivity to other systems. Lessons learned from terrestrial mines and shallow water systems *can* be incorporated into DSM environmental management, but attributes unique to the deep sea should be considered while assessing impacts and developing regulation. There are also important aspects of the regulatory framework that need to be implemented due to the international nature of relevant resources and ecosystems.

3. Ecosystem services associated with deep-sea mineral resources and their host habitats

Of the four primary mineral resources that are being considered for DSM, phosphorites occur primarily within national jurisdictions and are owned by nation states. Polymetallic sulfides, ferromanganese crusts, and polymetallic nodules occur both within national jurisdictions and in international waters. Those mineral resources in the Area are under the jurisdiction of the ISA and are considered the common heritage of mankind by the ISA in accordance with UNCLOS, Article 136 (UNCLOS, 1982; Jaeckel et al., 2016a). Article 140 further states that all activities in the Area should be done for "the benefit of mankind as a whole" (UNCLOS, 1982). By definition, ES contribute to human well-being, generating multiple values to society which include economic gains (e.g. from fisheries), social progress (e.g. education and art), and ecological sustainability (e.g. resilience and adaptation). Although ES valuation often includes economic and social indicators, ecological sustainability is rarely considered. In order to ensure benefits to mankind as a whole, all values must be factored into the development of DSM regulation by the ISA and ES provide a useful tool to do so.

The identification of stakeholders is an important step to developing DSM regulation that benefits mankind as a whole. The use of an ES framework can help identify relevant stakeholders through mapping tools and valuation studies. Stakeholders benefitting from provisioning services may differ from those benefitting from regulating or cultural services. Stakeholders may have different values with competing objectives, and the ES of concern may emerge at different spatial and temporal scales. For example, many provisioning services may happen at the scale of a vent or a seamount while regulating services emerge at larger, landscape spatial scales or long time scales, and can be more diffuse. Identifying stakeholders may facilitate independent review and public participation in environmental impact assessments for DSM (Lallier and Maes, 2016).

In its current and draft regulations, the ISA invokes the precautionary principle as outlined in the Rio Declaration, which states that a precautionary approach should be widely applied with scientific uncertainty as an invalid reason for delaying measures to prevent environmental degradation (Rio Declaration, 1992). The precautionary principle should be applied "as far as reasonably possible" by the ISA, sponsoring States, and DSM concession holders. What is needed is a clear articulation of the how the precautionary principle will be operationally implemented (Majone, 2002; Jaeckel, 2016b). There are a range of unknowns regarding the environmental impacts of DSM that need to be considered and ideally avoided before large-scale exploitation of deep-sea mineral resources begins (Nautilus Minerals Nuigini Limited, 2008; Schmidt, 2015).

3.1. Polymetallic sulfides

Polymetallic sulfides are found at hydrothermal vents where water circulates through oceanic crust, at spreading centers, back-arc basins, and volcanic arcs (Petersen et al., 2016). As the fluids are heated by magma, metals in the crust are leached into the water and expelled from black smokers, where they precipitate upon contact with cold seawater. These sulfides form large deposits of varying sizes and are rich in minerals including zinc, lead, barium, silver, and gold (Boschen et al., 2013). These have triggered a deep-sea "gold rush" (Ramirez-Llodra et al., 2011; Merrie et al., 2014; Petersen et al., 2016), but the profitability of these extractive activities is still being debated.

Vent communities can be dominated by large clams, mussels, snails, and siboglinid tubeworms (Ramirez-Llodra et al., 2007). These species produce carbonate shells and chitinous tubes (Ruan et al., 2008) that provide structure and create substrate at vent sites. This structural diversity can lead to more available niches and, ultimately, greater biodiversity (Govenar, 2010). The biogenic structures of these organisms may be used by non-vent fauna once the active flow ceases (Levin et al., 2016). There are also endemic vent fauna that further contribute to deep-sea biodiversity (Nakajima et al., 2015), which can influence ES. Tubeworm hemoglobin as a template for artificial blood (Flores et al., 2005) and unique armor inspired by scaly foot snails (Yao et al., 2010; Blaustein, 2010) are examples of how the faunal biodiversity might translate into provisioning services.

In addition to contributing to deep-sea biodiversity, vent microbial communities appear to play a key role in regulating services, such as the global cycling of carbon, sulfur, and potentially heavy metals (Jeanthon, 2000; Meyer-Lombard et al., 2013). Vents are areas of high productivity due to the presence of chemosynthetic microbes that transform and recycle carbon (Dubilier et al., 2008). Microbes and symbiont-bearing animals can consume sulfide, and methane (which could act as a greenhouse gas if released into the atmosphere) (Jørgensen and Boetius, 2007). Vent microbes hold potential for biotechnology advancement, particularly for industrial applications at high temperatures. Examples of thermophile applications include DNA polymerases for polymerase chain reaction (Terpe, 2013) and anhydrases for carbon dioxide scrubbing (Fig. 2A) (Mahon et al., 2015). Other applications include use of lipases, pullanases, and proteases for detergent, food processing and waste treatment. Amylases are used for baking and brewing, and xylanases and cellulases are used for pulp and paper processing and recycling (Leary, 2004). These biological compounds may provide provisioning services (new products), regulating services (global iron cycling (Tagliabue et al., 2010; Resing et al., 2015)), and cultural services. Unfortunately at this point in time, many if not most ES derived from vent microbes and organisms remain to be discovered or identified.

3.2. Ferromanganese crusts

Ferromanganese (or cobalt) crusts form as minerals precipitate out of seawater onto exposed hard substrate. These minerals include cobalt, nickel, platinum, thallium, and tellurium (Hein et al., 2000). Some of these rare metals are used for photovoltaic solar cells, hydrogen fuel cells, electric car batteries, computer chips, cell phones, and other technology (Hein et al., 2013). They are often found on seamounts throughout the global ocean, with deposits having the greatest commercial potential at 800–2500 m water depth (Yesson et al., 2011). Crust formation proceeds at very slow rates on the order of millimeters per million years (Usui et al., 2007).

Seamounts also provide hard attachment substrates used by sessile cnidarians and sponges to extend above the boundary layer (Hoff and Stevens, 2005; Schlacher et al., 2014), creating reefs or gardens and supporting a host of biodiversity (Auster et al., 2005; Cathalot et al., 2015). The coral and sponges also provide an ecological function (supporting service) in the form of nursery habitat (Baillon et al., 2012). The three-dimensionality of seamounts accelerates water flow and concentrates food particles, creating local areas of higher productivity and higher biodiversity relative to surrounding areas (Morgan et al., 2015). This high productivity provides provisioning services in the form of fish catch by attracting mobile organisms, such as commercially-fished orange roughy and oreo that aggregate around seamounts (Fig. 2B), as well as sharks, billfish, and other pelagic predators (Hughes, 1975; Koslow, 1997; Morato et al., 2010). Some organisms on seamounts provide templates for novel biomaterials. For example, bamboo corals are a model for synthetic human bone replacements (Ehrlich et al., 2006) and sponge spicules are superconductors for light (Brummer et al., 2008).

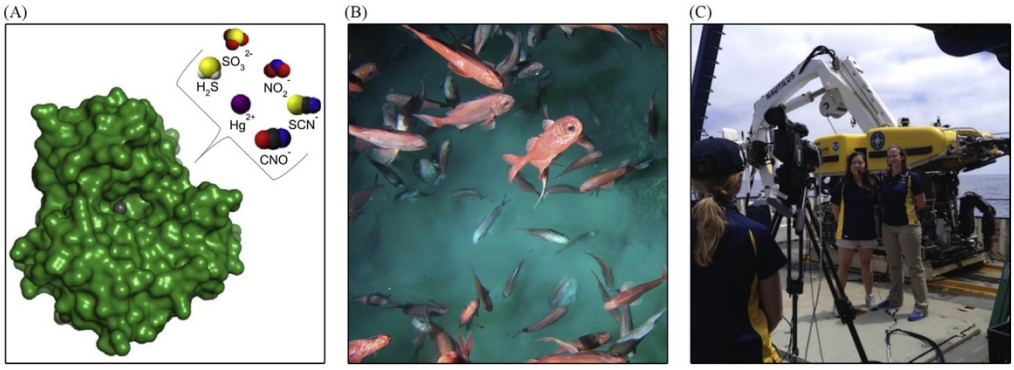

Fig. 2. Examples of deep-sea ecosystem services. (A) Provisioning service of industrial agents – Carbonic anhydrase from a hydrothermal vent bacterium proposed for industrial carbon dioxide scrubbing; image from Mahon et al. (2015). (B) Provisioning service of fish catch – A spawning aggregation of orange roughy (*Hoplostethus atlanticus*), a commercially-fished species, on the summit of a protected seamount at 890 m on New Zealand's Chatham Rise; photo courtesy of New Zealand's National Institute of Water and Atmospheric Research and the Ministry for Primary Industries. (C) Cultural service of education – A live-stream from scientists on the E/V Nautilus to K-12 students onshore, sponsored by Ocean Exploration Trust; photo courtesy of L.A. Levin.

3.3. Polymetallic nodules

Polymetallic (or manganese) nodules were first discovered in 1873 during the *H.M.S. Challenger* expedition. They are found on the abyssal plains beneath areas of low productivity, such as the eastern Pacific Ocean and the Indian Ocean (Petersen et al., 2016). Each nodule begins as a small, hard fragment of debris (e.g. tests or shells, shark teeth, other nodule fragments), and grows when dissolved metals precipitate on its surface. These metals include manganese, nickel, titanium, vanadium, cobalt, and iron, and are in increasing demand for modern electronic applications and green technologies such as thermal cooling devices and chemical sensors (Hein et al., 2013). Nodule formation is very slow; in the Pacific, growth is 1–2 mm per million years (McMurtry, 2001). Despite their slow formation, nodules can be found at densities greater than 10 kg/m^2 in the Clarion–Clipperton Fracture Zone in the eastern equatorial Pacific (Morgan, 2000).

The biological communities associated with nodule provinces are far less dense and have lower biomass relative to hydrothermal vents and seamounts, but host a greater diversity of infauna and epifauna including polychaetes, echinoderms, and crustaceans (Mullineaux, 1987; Howell et al., 2002; Brandt, 2005; Glover et al., 2001, 2015, 2016). A portion of these organisms show some degree of endemism, contributing to deep-sea biodiversity (Rex et al., 2005; Rex and Etter, 2010). The nodules provide hard substrate, creating available niches for specialized fauna (Thiel et al., 1993; Veillette et al., 2007; Vanreusel et al., 2016). In addition, the presence of hard substrate in an expansive area of soft sediment can be an important conduit for genetic resources (Janssen et al., 2015). The ES associated with nodule provinces may be related to the vast area where carbon is sequestered, and the high diversity of small, often rare eukaryotes with currently unknown functions and capabilities.

3.4. Phosphorites

Phosphorites are primarily found in shallow sediments on continental margins where upwelling occurs and surface production is high, such as the California, Humboldt, Canary, and Benguela current systems (Baturin, 1971; Föllmi, 1996). Due to low oxygen content in the upwelled waters, a substantial amount of organic matter reaches the sediment, setting the stage for phosphorite formation which is thought to be mediated by bacteria (Baturin, 1971; Schulz and Schulz, 2005). Phosphorite deposits are rich in phosphorous, calcium, and fluoride and are widespread on continental margins (Baturin, 1971).

There is current interest in mining phosphorite mineral deposits on the shelves and slopes of Namibia, South Africa, New Zealand, and Mexico. The phosphorite beds in these areas tend to be poorly characterized with respect to small biota and microbes, which may have value as genetic resources due to their unusual tolerance of extreme anoxic or sulfidic conditions. There is concern that DSM may make permanent changes to benthic systems that are vital for the reproduction, feeding, and survival of key species (Leduc et al., 2015). This concern appears to be particularly relevant because phosphorites have relatively low value, suggesting that large areas need to be mined in order for this type of DSM to be profitable. Continental margins with phosphorites support productive fisheries and are also subject to oil and gas drilling, shipping, and use by species with high conservation value, such as marine mammals and turtles (Findlay et al., 1992; Reeves, 2000; Campbell and Smith, 2006).

These four targeted mineral resources provide some similar ES: biodiversity (structure which contributes to genetic resources, potential for adaptation, and resilience), carbon sequestration (Feely et al., 2001), cultural services (Fig. 2C), and the unknown. For example, 188 natural products from marine fauna (found at depths ranging from 50 m to >5000 m) have been described since 2008, including compounds used to treat cancer and infectious diseases (Skropeta and Wei, 2014). Although these ES are shared, they are distinct among habitats, and perhaps even within the same type of habitat, i.e. for endemic species. For example, though vents and seamounts both contribute to deep-sea biodiversity, they cannot be substitutes for each other in the context of biodiversity because they host different communities of organisms. Each habitat provides a different magnitude of ES that operates on varying spatial and temporal scales, and will experience distinct impacts from mining.

The proposed mining process is reasonably similar across the different mineral resources. In general, the resource is cut (excluding nodules), aggregated, pumped to the surface, settled, and then excess sediment and seawater is expelled. Each of these processes affect ES through direct physical disruption, changes in substrate, light, noise, sediment plumes, smothering, release of contaminants, and changes in biogeochemistry (Oebius et al., 2001; Nautilus Minerals Niugini Limited, 2008; SPC, 2012). These effects will interact to change ES, altering productivity, connectivity, rates of extinction, and other characteristics of the ecosystem (Nautilus Minerals Niugini Limited, 2008; McClain and Barry, 2010; Van Dover, 2014b). Depending on the indicator used, it may be impossible to distinguish which specific impact of the production process is altering a given ES (e.g. sediment plume vs. contaminants vs. loss of source propagules).

4. Proposed framework and approach

Werner et al. (2014) uses the Gulf of Mexico to illustrate a practical approach to implementing an ES framework for the oil and gas industry. The authors suggest three steps: (1) prioritize relevant ES, (2) assess indicators of ES capacity, and (3) rank indicators to identify the most effective. The initial steps proposed below are loosely based on suggestions by Werner et al. (2014), with changes that adapt an ES framework to the context of DSM with a focus on valuation and implementation.

4.1. Identification of potential DSM impacts on ES

Deep-sea ES have been broadly described (Armstrong et al., 2012; Thurber et al., 2014). What is still unknown are which ES will be impacted by DSM and to what extent. Criteria for identification of vulnerable ES could include sensitivity to disturbance (from DSM and cumulative impacts), recovery and restoration potential, existence of possible substitutes, and synergistic effects on other ES. Although similar disturbances may result from extraction of the different mineral resources, each impact may manifest differently among habitats. Information about physiology and metabolism, dispersal and connectivity, nutrient and element cycling, and life histories is imperative to uncover details about structure, function, and, ultimately, the ES they provide. It is also important to acknowledge the strong likelihood for discovery of new ES. This is something rarely considered in decision-making and is of lesser concern with more widely studied ecosystems where there is a long history of human activity.

4.2. Consideration of the role of ecological functions (supporting services)

By definition, ecological functions (supporting services) are necessary for the provision of final ES (de Groot et al., 2010). They derive from structural characteristics of the ecosystem (Table 1), and need to be identified and protected in order to preserve ES.

Table 1
Measurable ecosystem structures and ecological functions (supporting services) that support ecosystem services.

Service	Function (Supporting service)	Structure
Provisioning Services		
Fish catch	Breeding or spawning grounds	*Physical structure Adult distribution *Population density/biomass Population age structure
	Nursery habitat	*Physical structure *Flow regime Biotic structure/ecosystem engineers Egg, larval, and juvenile abundance Prey abundance
	Refugia	*Physical structure *Flow regime *Population density/biomass Biotic structure/ecosystem engineers
	Secondary production	Feeding locations
	Trophic support	*Organic matter flux (e.g. via sediment traps) *Benthic community composition Prey abundance Food web structure (e.g. via *stable isotope analysis, gut content analysis) Growth rates
	Dispersal *Connectivity	*Flow regime (e.g. via passive transport models) *Hydrography Endemicity Life-history traits Larval distribution, temporal patterns Larval density
Pharmaceuticals Industrial agents Biomaterials	Biodiversity	*Physical structure *Hydrography *Faunal characterization (e.g. mega, macro, meio, protozoa) *Microbial characterization Genetic diversity
	Metabolic activity	Physiology *Water chemistry Natural products chemistry *Microbial characterization Microbial transcriptomics & metabolomics Faunal metabolomics
Regulating Services		
	Surface photosynthesis	*Phytoplankton density/biomass Photosynthetic pigments Nutrient concentrations
	Chemosynthesis	*Water chemistry *Microbial characterization Symbiotic relationships
	Remineralization	*Microbial characterization Phaeopigments *Water chemistry
	Carbon flux	*Plankton community composition
	Bioturbation Bioirrigation	*Sediment properties (e.g. grain size, C_{org}) *Sediment community composition Sediment Radiochemistry: Pb-210, Th-234 *Pore-water chemistry
Climate regulation – methane sequestration	Aerobic methane oxidation	*Water chemistry (e.g. methane concentration) Methanotrophic bacteria
	Anaerobic methane oxidation	*Water chemistry (e.g. methane concentration, sulfate concentration) Methanotrophic archea characterization Sulfate-reducing bacteria characterization Authigenic carbonates
Climate regulation – greenhouse gas regulation	Nitrogen fixation Nitrification Denitrification Nitrate reduction	*Organic matter flux *Nutrient concentrations in water and pore-water *Pore water chemistry
	Ammonium oxidation	*Microbial characterization
Biological control of populations		Pest density/biomass & distribution Predator density/biomass & distribution Viral abundance Food web structure
Waste absorption	Assimilation Metabolic activity	*Water chemistry *Baseline level of toxins (e.g. trace metals, polycyclic aromatic hydrocarbons) Physiology *Faunal characterization *Microbial characterization
	Bioturbation Bioirrigation	See above.
Cultural Services		
Educational Aesthetic including the arts Existence Stewardship	All functions, and subsequently structures, contribute to some aspect of cultural services. However, how these services are perceived and prioritized are dependent on factors such as cultural background and socioeconomic status, as well as the communication of deep-sea science and issues (e.g. via news articles, visuals).	
Other ecological functions		
	Element and nutrient cycling Iron oxidation Manganese oxidation Sulfur oxidation Sulfate reduction	*Trace element concentrations in water and sediment *Nutrient concentrations in water and pore-water *Pore water chemistry *Microbial characterization

Structures and functions annotated with an asterisk (*) are explicitly included in the International Seabed Authority environmental impact assessment recommendations for exploration for seabed minerals in international waters (ISA, 2013a).

The types of information and measurements necessary to identify and quantify relevant function vary among different deep-sea ES. For example, those necessary to provide fish and invertebrate catch include breeding or spawning grounds, nursery habitat, primary production for trophic support, and refuge from predators. Information regarding life histories is especially important in order to quantify and valuate the final ES, as well as to effectively manage stocks (Adams, 1980; Shuter et al., 1998). Identifying crucial habitat, estimating survival and recruitment rates, and linking larvae and juvenile populations to adult populations are necessary to translate biological measurements into economic value (Botsford et al., 2009). Another example includes carbon sequestration, which is influenced by functions such as primary productivity (Kuypers et al., 2002), carbon flux to the bottom (Jahnke, 1990), degradation and burial rate of organic carbon (Hartnett et al., 1998; Breithaupt et al., 2012; Smith et al., 2015). These measurements will help estimate the capacity for deep-sea

carbon sequestration, which can then be used for valuation studies and mitigation planning. Information regarding deep-sea functions can be used to develop ecosystem principles, which become an educational element in ES valuation (Jobstvogt et al., 2014a). However, lack of data and knowledge regarding deep-sea structure and function often makes full characterization challenging.

Some functions (supporting services) may be an input to multiple final ES. For example, production can influence fish and invertebrate catch via trophic support, and carbon sequestration via subsequent export to depth. Both the direct and indirect impacts, as well as downstream consequences of DSM may affect the ability of targeted habitats to provide ES through channels still unknown. These knowledge gaps invoke the use of a precautionary approach, and may in some instances suggest the postponement of large-scale DSM until these relationships are better understood.

4.3. Developing ES indicators

Practical ES indicators need to be developed as requirements for baseline data collection and monitoring programs. Werner et al. (2014) establishes criteria to assess ES indicators. Lagging indicators detect ES changes after they occur and are useful in establishing the level of impact on a service. Leading indicators provide information about structure, function, and potential sources of change. Structures and functions themselves may serve as indicators of ES (e.g. Table 1). For example, water turbidity and flow regime may foreshadow impacts from sediment plumes caused by DSM (e.g. suffocation, preventing larval settlement (Jones, 1992)). Indicators should be practical, sensitive, and easy to monitor in order to facilitate implementation into baseline data collection and monitoring programs. Different indicators for the same ES may be more practical in one setting versus another. For example, indicators for carbon sequestration may differ between a nodule province and a phosphorite-rich margin due to the large difference in area over which carbon is sequestered. One might rely on satellite-based surface chlorophyll measures to integrate over large areas while the other could use time-series sediment trap data; both would involve radioisotope-based sediment accumulation measures. Indicators should be quick to respond to changes in ES. The measurements should be taken accurately using a standard protocol over spatially-relevant scales in order to statistically analyze the obtained data. Consistent methods and reliable data are important inputs for making good policy decisions and are convincing to policymakers. Once ES indicators are developed, they can be used to monitor changes in ES and their value (Boyd et al., 2014).

4.4. Valuation of ES

The literature on valuation of environmental goods and services is large (Hanley and Barbier, 2009), but its application to the deep sea has been extremely limited (e.g. Table 2). Wattage et al. (2011) and Jobstvogt et al. (2014b) attempt to estimate the value of deep-sea corals and biodiversity, respectively, using stated preference methods that ask individuals for information related to their economic value for a non-market good or service (in contrast to revealed preference methods that infer values from consumer and firm behavior). Both studies ran into challenges. The public lacks knowledge about the deep sea, but more important, scientific uncertainty is sufficiently large that it is difficult to comprehensively describe changes in ES in a way that is readily understandable to a lay audience. From a stated preference perspective, it is possible to describe a policy that changes one or more ES, and ask the public to value that policy. However, it is important to realize that only the ES in the survey will be valued. The ecosystem

Table 2
Valuation methods typically used for different categories of ecosystem services

Service, function, or structure	Typical valuation method	Example
Provisioning Services Fisheries Pharmaceuticals Industrial agents Biomaterials	Market value Avoidance cost	1. **Market value of pharmaceuticals** (Erwin et al., 2010) 2. **Market value of coldwater coral fisheries** (Foley et al., 2011) 3. **Market value of fisheries** (Martin et al., 2016)
Regulating Services Climate regulation Biological control Waste absorption	Avoidance cost Replacement cost Production function approach Hedonic pricing Contingent valuation	1. Avoidance cost of carbon dioxide (Beaumont et al., 2008) 2. Avoidance cost of biological regulation (Zhang and Swinton, 2012)
Cultural Services Aesthetic Educational Existence Stewardship	Hedonic pricing Contingent valuation	1. CV of bequest value (O'Garra, 2009) 2. **Value of scientific investment** (Godet et al., 2011) 3. Choice modeling of stewardship value (Lim et al., 2015)
Ecological Functions (Supporting Services) Element/nutrient cycling Productivity/respiration Metabolic activity Habitat provision Bioturbation & C burial Dispersal/connectivity Biodiversity (ecosystem structure)	Production function approach Contingent valuation	1. **CV of coldwater corals** (Glenn et al., 2010; Wattage et al., 2011) 2. **CV of biodiversity** (Jobstvogt et al., 2014b)

Examples specific to the deep sea are in bold. CV is an abbreviation for contingent valuation.

principles approach presented by Jobstvogt et al. (2014a) offers an expert consensus approach to development of principles that link function (supporting services) to service, for use in educating the public prior to a valuation survey.

The estimated value of an ES is highly dependent on the population being sampled and survey scenario. Preferences for particular policies often differ systematically by age, culture, education, environmental attitudes, gender and race. This may lead to different sample populations placing different values on the same ES. How to aggregate value across individuals is well defined in a national context but it is in a nascent state for international resources managed by an international authority like the ISA.

Thinking about implementing a contingent valuation survey raises questions about whether maximum willingness to pay to avoid harm or minimum willingness to accept compensation to agree to the harm is the more appropriate property rights framework. Because the ISA has no ability to tax the public for DSM, the willingness-to-pay mechanism would take the form of higher prices in return for implementing DSM in a manner that is less harmful to the environment. The common heritage of mankind language suggests that minimum willingness to accept compensation is appropriate, but this property right is difficult to reliably implement. How the ISA distributes any revenue can influence this interpretation.

Revealed preference approaches include: (a) using the price of a resource bought and sold in a market, (b) estimating the cost of averting behavior related to an adverse change in an ES, (c) determining the replacement cost of the next best option, (d) estimating how the output of production changes with changes in inputs including ES, and (e) estimating how the price of a marketed product changes as attributes of that product (including

Box 1–Economic valuation methods and examples of potential application to the deep sea.

Market value: Market products are associated with a market and price that reflect their value, e.g. the value of a deep-sea fish species is its market value. These prices should be adjusted for any market imperfections, such as subsidies and barriers to entry.

Averting behavior (avoidance cost): How much is spent to avoid adverse changes in an ES, e.g. a fishing boat may incur extra costs to avoid areas where DSM is taking place.

Replacement cost: How much it would cost to replace an ES with the next best option for providing the same service, e.g. a climate change agreement might require the carbon not buried due to DSM to be sequestered through another channel, which has an associated cost.

Production function approach: Output from a production function depends on its inputs including different ES. The value of a final ES can be determined by how it influences the production output when that output can be valued in economic terms. For example, reducing pollution may increase the growth of a fish stock sold in the marketplace.

Hedonic pricing: The price of a good is seen to be a function of the bundle of its attributes (including ES) of which it is comprised, e.g. price differences between marketed fish with and without traces of DSM contaminants are related to the value of contaminant removal.

Contingent valuation, including choice modeling (CV): A stated preference method that involves surveys regarding maximum willingness-to-pay or minimum willingness to accept compensation for a non-market good or service, e.g. an individual could be asked how much they would be willing to pay to implement a program that protects one or more rare deep-sea species found in nodule provinces.

Benefit transfer: A method of transferring values estimated in a primary study, using one or more of the techniques above, to a similar system. It is often applied to many ES. For example, a study calculating the value of nursery habitat provided by a shallow-water coral reef might be used to estimate the same ES in a coldwater coral reef. Due to the unique nature of the deep sea, there are probably few cases where transfer from shallow water or terrestrial examples makes sense.

ES) are changed (see Box 1 for more details). The major problem with using most of the revealed preference approaches to assess economic impacts of changing ES on consumers and producers is the ability to quantify *all* of the important ES. We are able to identify many (but not all) deep-sea ES and have only just begun to develop methods to quantify them at the level of detail needed for economic valuation purposes.

4.4.1. Other social impacts

There are other social impacts that may need to be taken into account in addition to changes in economic value. Social metrics might include the number of people whose livelihoods depend on a given ES (e.g. fishermen) or the number of people who directly benefit from an ES (United Nations, 2016). These types of measurements may be particularly appealing to policymakers who are concerned with the distribution of policy outcomes and social equity.

4.5. Incorporation of ES into environmental planning and implementation

Significant advances in the environmental planning process are needed before large-scale commercial DSM commences. The following section outlines several steps within the environmental planning and implementation process where ES approaches can be included (Fig. 3), and provides recommendations for the operationalization of ES concepts. As strategic environmental assessments provide a big-picture look at policies and programs, ES mapping could provide a useful tool for assessment of multiple services as well as multiple stressors. Environmental impact assessments are more specific; they look at an activity, involving baseline data collection and monitoring programs that should include measurements to help characterize how ES indicators are changing (Table 1). ES can also be incorporated into environmental management plans that outline methodologies to be used over the course of the activity, by serving as criteria for prioritizing areas for spatial protections and for defining ecological thresholds. The application of ES concepts may be most integral to the development of a mechanism in which the value of lost ES can be used as a measure of the compensation required for damage to the environment.

4.5.1. Strategic environmental assessments

The earliest activities in environmental planning and management include a strategic environmental assessment (SEA), which is the "formalized, systematic, and comprehensive process of evaluating the environmental effects of a policy, plan, or program and its alternatives" (Therivel and Partidario, 1996). SEAs consider all existing activities and human uses, and differ from EIAs in that EIAs generally consider one site-specific activity rather than a policy, plan, or program. Partidario and Gomes (2013) suggest ES incorporation into SEA methodology with three main steps: (1) identify ES and stakeholders, (2) prioritize ES, and (3) perform an ES assessment. It is important that the first and third steps consider appropriate scales. DSM stakeholders may range from individual firms to regional beneficiaries with varying degrees of knowledge and investment. For the third step, spatial and temporal scales will differ greatly among the resources and settings of interest. For example, space and times scales for delivery of climate regulation services in nodule provinces are expansive, whereas services related to fisheries on seamounts may be highly localized. Active hydrothermal vent communities may be able to recover on year to decadal time scales (Tunnicliffe et al., 1997; Van Dover, 2010), but fauna associated with inactive hydrothermal vents, cobalt crusts, or polymetallic nodules could take much longer to recover (Thiel et al., 2001; Smith et al., 2008).

Mapping tools can be used to examine spatial distribution of ES, analyze synergies and tradeoffs between ES, compare ES supply and demand, and prioritize areas for conservation (Maes et al., 2012). There exist many examples of ES mapping (e.g. Raudsepp-Hearne et al., 2010; O'Farrell et al., 2011; Burkhard et al., 2012), but few are from marine systems (e.g. ; Mangi et al., 2011), and none are from the deep sea. Challenges to marine ES mapping include dynamic benthic and pelagic habitats over time, and poor understanding of the processes that occur in them (Maes et al., 2012). The problem of lack of data is even more prominent in the deep sea and highlights the importance of baseline data collection and monitoring requirements. Marine ES mapping can provide a tool to identify areas that may be especially valuable or vulnerable and ensure the proper environmental protections are in place as human activities in the deep sea expand.

4.5.2. Environmental impact assessments

The ISA requires environmental impact assessments (EIAs) in their exploration contracts in order to evaluate the risk to the environment, socio-economic outcomes, cultural resources, and human health (ISA, 2010a, 2010b, 2010c). Based on these assessments, strategies and methods can be proposed to avoid or minimize the likelihood or severity of potential hazards. Current ISA exploration regulation states that activities cannot be

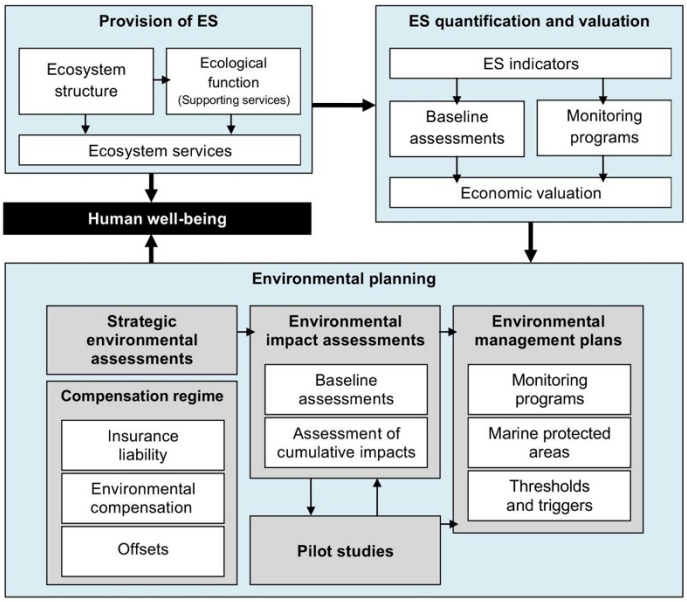

Fig. 3. Relationships among components of ecosystem services, their study, and phases in environmental planning where ecosystem services could be incorporated.

undertaken if there is evidence indicating risk of "serious harm to the marine environment," i.e. the ISA and its concession holders must prevent activities that present "significant adverse changes" (ISA, 2010a, 2010b, 2010c). However, the definition of "serious harm" is under debate (ISA, 2015). ES could serve as one standard for assessing serious harm in the context of DSM EIAs because they link environmental health to human well-being. One recommendation is for EIAs to characterize ES provided by the area of concern, the structure and function necessary to maintain those services, and potential DSM impacts on them. Evaluating DSM impacts on ES will help minimize loss of valuable environmental benefits.

4.5.2.1. Baseline data collection and monitoring programs. EIAs require acquisition of baseline data and the proposal of a monitoring plan (ISA, 2013a). Baseline data collection should include physical, chemical, and biological measurements that serve as ES indicators (which may include measurements of structure and function) or inform about ES to characterize the targeted habitat and its services (see Table 1). Examples of these measurements may be bioturbation rates, respiration rates, and sedimentation rates, which affect carbon sequestration (Vardaro et al., 2009), or concentration of fish larvae of commercial species in the water column (Werner et al., 2014). Deep-sea scientists can play a major role in adjusting current baseline data acquisition practices to better reflect ES by developing shared protocols for characterizing and quantifying ES. It will also be important to standardize these protocols across SEAs, EIAs, and other assessments to produce a better understanding of DSM impacts on ES. ES must first be adequately characterized in order to observe any changes. The establishment of baseline ES provision is necessary to monitor how DSM will affect the natural processes that contribute to human well-being.

When adverse ES changes are observed, compensation for any value lost should be collected by the ISA. How much of this compensation should be provided to major stakeholders who are adversely impacted versus the general public is an open question. Should DSM impacts improve deep-sea provision of some ES, value added could manifest as environmental credits to the concession holder. Current baseline and monitoring measurements recommended by the ISA do not explicitly include ES, but do contain measurements to characterize habitats and biodiversity (see Table 1). Translating these measurements into final ES remains a challenge. In part, this is because DSM can set in motion multiple complex changes. For instance, disturbance of an ES can facilitate the entry of an invasive species, which can potentially increase local biodiversity in an undesirable way, creating an ecosystem disservice or a new or enhanced service that has negative consequences (Zhang et al., 2007).

The first several pilot studies and commercial DSM projects need to be treated as an opportunity to do extensive monitoring in order to determine the effectiveness of ES Indicators, to identify the ES influenced by DSM activity, and to examine the magnitude of impacts. There is a clear learning-by-doing aspect, an economic concept in which practice yields higher efficiency (Ying, 1967) that can help inform future DSM regulation and activity by incorporating the results from these initial studies and projects. An interesting question here is how much of this learning should be paid for by the DSM concession holders, sponsoring nations, and the ISA as the knowledge gained will make future DSM projects easier to assess.

4.5.2.2. Assessment of cumulative impacts. The number of anthropogenic impacts on the deep sea is increasing as commercial interest grows and greenhouse gas emissions continue (Ramirez-Llodra et al., 2011). These may impact deep-sea ES in additive, antagonistic, or synergistic ways (Crain et al., 2008). Mining claims could be made in areas that are ecologically connected or are subject to deep-sea fishing, shipping, waste disposal, pollution, or major climate change impacts (Mengerink et al., 2014; Levin and

Le Bris, 2015). A systematic examination of cumulative impacts on ES could be incorporated into EIAs by determining the impacts of different combinations of multiple mining events, different types of mining, direct human activity, and climate change. There could be spatially disjoint impacts from different stressors acting on different ontogenetic stages of major fishery species or endangered species, which only matter when combined. DSM regulation needs to reflect the possibility of cumulative impacts from multiple mining events (e.g. at multiple claims in the Clarion-Clipperton Fracture Zone and the Mid-Atlantic Ridge), and from non-mining activities that cause more significant changes in ES relative to impacts from DSM alone. This may mean whole suites of ES, ecological functions (supporting services), or ecosystem structures must be protected in order to maintain ES of interest (Koch et al., 2009). One suggestion to minimize cumulative impacts on ES could be to incorporate an ES supply function into existing tools that map cumulative impacts (e.g. Halpern et al., 2008). This could provide insights on what areas may be most valuable to protect and most vulnerable to impacts.

4.5.3. Environmental management plans

The draft ISA regulation for commercial DSM will require an environmental management plan (EMP) that outlines methodologies; sampling and archiving before, during, and after operations; measureable criteria; and threshold indicators (ISA, 2015). Incorporation of ES into these aspects of an EMP provides a mechanism to take into consideration the societal value of natural processes.

4.5.3.1. Marine protected areas. ES has a role to play in the identification and designation of areas that are to be protected from mining impacts (e.g. Chan et al., 2006; White et al., 2011). There are several different categories of protected areas being considered in the context of spatial management for DSM. The United Nations defines vulnerable marine ecosystems (VMEs) as populations, communities, or habitats that are "both easily disturbed and very slow to recover, or may never recover" (FAO, 2009a). These VMEs currently include hydrothermal vents (e.g. Reyjkanes Ridge) and seamounts (e.g. Koko and C–H seamounts in the Pacific), and are to be protected from significant adverse impacts (FAO, 2009b). The ISA recognizes VMEs and has regulations in place to prevent serious harm to them (ISA, 2013b). In addition, the ISA has designated large sections in the Clarion–Clipperton Fracture Zone polymetallic nodule province as areas of particular environmental interest (APEIs) (ISA, 2011; Wedding et al., 2013), but has not yet done so for other mineral resources. There is no standard protocol for identifying VMEs or APEIs; the definition for VMEs is broad but includes criteria such as uniqueness or rarity, functional significance of the habitat, fragility, life-history traits of component species that make recovery difficult, and structural complexity (FAO, 2009a; Auster et al., 2011). ES can serve as one standard for designation. For example, it may be possible to discern areas of high aggregate ES value and identify them as VMEs when that value is above a specified level.

The United Nations Convention on Biological Diversity (CBD) has its own form of spatial protection called ecological or biologically significant area (EBSAs), which are defined as "geographically or oceanographically discrete areas that provide important services to one or more species/populations of an ecosystem or to the ecosystem as a whole" (CBD, 2008). EBSAs must meet the following criteria: uniqueness or rarity, requirement for survival, endangered or threatened species occurrence, vulnerability, fragility, productivity, diversity, and naturalness (CBD, 2008). The CBD has identified hydrothermal vents and seamounts throughout the global ocean as EBSAs (e.g. Juan de Fuca and Guaymas Basin hydrothermal vents, Atlantis seamount in the Indian Ocean), and indicates they should be managed in a way that conserves their integrity, which includes creating MPAs recognized by international law (CBD, 2008).

Within ISA mining claims, there are also other potential protections that can be allocated to maintain ES. These may include unmined reference sites, voluntary permanent or temporary unmined areas, or areas turned back to the ISA after prospecting or exploring. Protected areas should be sites that would have otherwise been mined in order to be effective and of value. One criterion for identifying protected sites could be the potential to replace or provide similar ES as those lost or damaged at the mined site.

The supply and demand of ES and their value can also be mapped spatially, providing a useful tool for marine spatial planning (Naidoo et al., 2008; Burkhard et al., 2012). Areas of high ES value should be considered for protections against mining-related activities that may decrease that value, with close attention to where the value would be lost and where the beneficiaries are. A map of ES demand can be used to facilitate equitable distribution of natural capital as the common heritage of mankind.

4.5.3.2. Thresholds and triggers. Accurate baseline data and monitoring during the exploratory phase may allow for the identification of environmental thresholds or triggers. An ecological threshold is a "tipping point" at which ecosystem conditions undergo a rapid and possibly irreversible change exceeding normal ranges (Groffman et al., 2006). If we know a threshold will be reached due to mining impacts in a given area, then that area may require spatial protections. Once mining begins, small losses of ES may be acceptable, but there may be thresholds or triggers that would require cessation of activity. The concept of a threshold can also be applied to ES as the point at which ES are no longer provided on a significant scale (Koch et al., 2009). Often services do not accrue or decline linearly (Barbier et al., 2008). This might come from unexpected impacts to ES, such as the cumulative effects of DSM and climate change. Identifying thresholds requires an established baseline and knowledge about natural variability and, therefore, may prove especially difficult in the deep sea where there is a lack of data. Identifying ES thresholds may involve percentage losses of foundation species, such as those with chemosynthetic symbionts, or of habitat known to support a commercially-fished population. Another example could be a sediment plume that extends above a certain water depth that may disrupt shallow-water and vertically-migrating communities.

4.5.4. Environmental damage compensation regime

ES can play a role in the development of a DSM environmental damage compensation regime. Current ISA exploration regulations make DSM concession holders liable for any damage to the marine environment from their activities and require them to maintain proper insurance (ISA, 2010a, 2010b, 2010c). There is discussion of an environmental liability trust fund as well as a seabed sustainability fund which would fund research on best environmental practices and the effects of seabed dredging (ISA, 2015, 2016). However, these are not yet developed.

In terrestrial mining, firms are responsible for the release of any hazardous substances into the environment. For example, the U.S. Superfund, or Comprehensive Environmental Response, Compensation and Liability Act of 1908 (CERCLA), gives federal land managers the authority to demand response and cleanup funds for contaminated mining sites (Seymour, 2004). Superfund sites are generally waste sites that pose a risk to human and environmental health. ES are not specifically mentioned in the terrestrial mining legislation but they have increasingly been the focus of restoration efforts. The U.S. has a program called the Oil Spill Liability Trust Fund, which is funded by petroleum taxes, environmental fines, and compensation for damage to natural resources (26 U.S. Code §

9505). The fund is used to quickly respond to accidents and emergencies (Continental Shelf Associates, Inc., 2006), although it is not always sufficient for very large injuries (e.g. Deepwater Horizon).

The ISA is an international body with little power or capacity to tax its member states. However, there is discussion of royalty payments to the ISA (and how they may change over time in response to increasing concession holders, decreasing costs, or other economic factors) as contribution to the common heritage of mankind. Seabed minerals found in international waters are considered the "common heritage of mankind" (UNCLOS, 1982). ES related to the same waters should be treated similarly because they can provide benefits to society as a whole. Known short- and long-term damages to and loss of ES caused by DSM should be internalized in order to reflect the total social cost of DSM. In order to set an effective environmental damage compensation regime, the lost economic value associated with diminished ES requires reasonably accurate quantification. The incorporation of ES into baseline data collection, pilot mining tests, and monitoring programs can help calculate the value of lost environmental benefits and that value can be used to create an efficient compensation regime.

The revenue raised from compensation for lost ES and environmental damage can be used to compensate stakeholders (once identified) including the general public, to fund the creation and enforcement of MPAs, to restore impacted sites (if and where possible), or for scientific research that improves environmental management of the deep-sea environments being altered. The draft ISA regulation for commercial DSM includes a sustainability fund to direct further research and develop technology (ISA, 2016). It could be financed with environmental damage compensation payments and with any patent royalties from previously funded research.

5. Knowledge gaps relevant to identification and quantification of ecosystem services and potential tools to fill them

5.1. Linkages between structure, function, and service

ES are provided by ecological functions (supporting services) that are supported by ecosystem structure (Fig. 1; Table 1) (Kremen, 2005; Barbier et al., 2011; Thurber et al., 2014). Knowledge of the linkages among structure, function, and service are essential to predicting DSM impacts and calculating losses in ES and their value. In order to quantify and valuate ES, the mechanisms by which they are provided must be known. This could include fluxes of nutrients, metabolic rates, behavior, natural variability, and drivers of change.

Perhaps the biggest anticipated impact of DSM on the marine environment is loss of biodiversity. Biodiversity has been shown to increase function in the deep sea (Danovaro et al., 2008), and therefore, a loss of biodiversity can potentially result in the loss of ES. The ISA requires DSM concession holders to collect baseline data and monitor any DSM impacts on the marine environment (ISA, 2010a, 2010b, 2010c). Representative fauna and dominant species of all size classes from a variety of habitats, including the water column, are required for such an assessment and must be sufficient to characterize the biodiversity of deep-sea habitats. However, maximizing biodiversity is not the same as maximizing ES or function. There are often tradeoffs between biodiversity and ES. In wetlands for example, there are nonlinear relationships between species richness and primary productivity, and consequently carbon sequestration (Barbier et al., 2008; Naidoo et al., 2008; Bene et al., 2011). The contribution of biodiversity to ecological function (supporting services) and provision of services is still largely unknown in deep-sea systems. Without further knowledge regarding these relationships, it remains difficult to translate these recommended measurements into achievement of better ecosystem health.

The concept of ES is rooted in terrestrial systems. The deep sea, in contrast, tends to have less clearly defined boundaries and may need novel approaches in order to understand and implement deep-sea ES as a guiding framework (Jobstvogt et al., 2014a). Next-generation genetic tools (e.g. next-generation sequencing, use of environmental DNA) can potentially provide a more complete picture of deep-sea biodiversity and also inform on its contribution to ES, particularly in regards to microbial nitrogen cycling, carbon fixation, and other regulating services (Baker et al., 2013; Gibson et al., 2015). Transcriptomics, proteomics, and metabolomics can identify biochemical pathways that may reflect functions (supporting services) linked to global element cycling, or may illuminate novel attributes that can lead to industrial applications (Skropeta and Wei, 2014). Although it can be difficult to interpret data generated from genetic tools due to lack of knowledge, their use may identify dominant taxa (e.g. Dell'Anno et al., 2015) that are important to ES. Biological traits analysis is another tool that can potentially reveal linkages among structure, function, and ES, transcending taxonomic differences among regions or ecosystems. This method statistically analyzes the relationship between multiple biological traits and environmental processes or parameters (Bremner et al., 2006). For example, the abundance of burrowing fauna can influence a benthic system's capacity to transport and store organic matter, nutrients, and contaminants (Constable, 1999; Reise, 2002). Application to the deep sea may prove challenging due to the lack of data on life histories (e.g. reproductive mode, larval survival rates) and behavior (e.g. feeding mode, vertical or horizontal migration). However, as more data are collected, biological traits can provide insight into environmental variability and function indicators, and more effective marine protected area designation (Frid et al., 2008; Mitwally and Fleeger, 2016).

5.2. Life histories, ranges, and genetic connectivity

Information about key species associated with ES is crucial to identifying ES and minimizing DSM impacts on them. For example, in Namibia mining impacts in phosphorite beds may remove ecosystem engineers (e.g. sponges, sea pens) or degrade nursery habitat for commercially-fished species (e.g. monkfish, hake, or their forage species, the bearded goby), resulting in decreases in fisheries landings. New research is needed to examine life histories of key species, including geographic dispersal, range, and ontogenetic changes in habitat to illuminate the linkages necessary for the provision of ES. Genetic connectivity among habitats must also be researched for insight into potential recovery times or probability of extinction. Areas with higher genetic connectivity may recover their biological communities more quickly. Patterns of gene flow and connectivity can also be helpful tools in designating "set-asides" (protected areas that support biodiversity and connectivity lost at the mining site) and unmined reference sites (Boschen et al., 2016).

New technology and instruments, such as the SentrY Precision Robotic Impeller Driven sampler (Billings et al., in press), can increase sampling capacity over larger spatial scales, longer time periods, and more types of samples (i.e. larvae). These sampling capabilities can lead to insight into larval dispersal, species ranges, and habitat-specificity of different life stages in the deep sea. The use of autonomous and remotely-operated vehicles is also helpful to better understand deep-sea processes. In particular, high-definition pictures and videos allow for visual surveys and observations that can help identify ES. The actual visualization of deep

habitats may reduce current sampling biases by allowing scientists to observe organisms, like mobile fish, that can avoid capture.

5.3. Spatial and temporal scales

Deep-sea ecological functions (supporting services) and the ES they support operate over a large range of spatial and temporal scales. Data collection and monitoring must reflect function-specific scales in order to accurately evaluate them. Furthermore, assessments must account for potential synergies among deep-sea functions. Interactions among functions are difficult to study when there is still an incomplete understanding of the deep sea and its habitats. For accurate ES assessment, new studies are needed addressing how deep-sea habitats change over space and time, and their interactions with the ecosystems of the surrounding seafloor and overlying water column, and with global geochemical cycles (Levin et al., 2016).

5.4. Recovery of structure, functions, and services

Deep-sea ES are dependent on ecological functions (supporting services) that will be affected by DSM (Glover and Smith, 2003; Clark et al. 2010; Van Dover, 2010). Research is needed under realistic conditions to determine whether biological communities will be able to recover from these impacts and, if possible, the time it would take to return original ES (Van Dover et al., 2014a). Resilience measures, such as recovery rates and thresholds (Mumby et al., 2014) that are sensitive to both spatial and temporal scales of the DSM activity and its impacts, need to be developed. This information is what participants in a contingent valuation survey will need to know in order to make an informed decision (Mitchell and Carson, 1989). Thresholds that are impossible to reverse may be reached. Rather than discover these thresholds after the fact, there is an opportunity to identify them before the start of DSM.

5.5. Economic valuation of ES

ES provide a tool that links ecosystems with human well-being, which then allows for economic valuation of these benefits. However, valuation has proven difficult to do accurately in the deep sea due to the lack of an adequate information base (Wattage et al., 2011; Jobstvogt et al., 2014a, 2014b). As a result, the data collection required by concession holders is essential to better understanding deep-sea ES. Habitat-specific measurements should also be included to monitor unique characteristics (e.g. sulfur recycling at hydrothermal vents). The data required to put values on ES not only include magnitudes (including how they vary over time and space), but also measurements of how ES are used by people and their perceptions of what the deep sea contributes to their well-being. Using an ES approach requires interdisciplinary collaboration between the natural and social sciences that may result in novel approaches and techniques in order to accurately quantify and value ES.

5.6. Definitions of terms

ISA regulation must be consistent with the principles set forth by UNCLOS (e.g. seabed minerals found in international waters must be treated as the "common heritage of mankind"), but interpretation and definition of terms remains a challenge. Current exploration regulation states that the ISA cannot approve any activities that might pose "serious harm" or "significant adverse change" to the marine environment (ISA, 2010a, 2010b, 2010c). However, the definitions of "serious harm" and "significant adverse change" are still points of debate. ES can serve as one measure for identifying serious harm, e.g., if an activity will result in the loss of ES sufficient to affect mining decisions. The definitions of these terms are likely dependent on how a healthy deep-sea habitat is defined and exactly what it is we want to protect (e.g. biodiversity).

Similarly, existing exploratory regulation calls for a precautionary approach (Rio Declaration, 1992), but how to operationalize such an approach is still ambiguous. Under the National Environmental Policy Act in the U.S., the Council on Environmental Quality has created a mitigation hierarchy: avoid, minimize, rehabilitate, and offset (CEQ, 2005). We recommend that these activities directly incorporate ES. While monitoring ES and mitigating for adverse impacts to them may help, practical implementation is likely to be complicated. The potential for rehabilitation and restoration in the deep sea is unknown (Schriever et al., 1997; Van Dover et al., 2012), but offsets are a major topic of current discussion. ES offsets should at the very least replace the same ES, provide a similar magnitude of benefit in as close geospatial proximity as possible, and serve the same stakeholders.

6. Conclusion

Incorporation of ES into international DSM regulation is a reasonable goal that will foster sustainability objectives. There is a single regulatory agency to consult (the ISA), commercial mining has yet to occur so there is an opportunity to set desirable precedents, and the quantification of ES will greatly facilitate the operationalization of a compensation regime that provides payment for environmental harm. Within national jurisdictions, the challenges may be greater as there are at least 150 nations with deep seabed and deep resources. For all, challenges to adopting an ES framework include the development of new knowledge needed to accurately quantify and valuate ES, and of optimal indicators of ES. New technologies and techniques, such as next-generation genetic tools, biological traits analysis, and novel robotic sensors can potentially help address these challenges. Illuminating the linkages among physical, chemical, and biological structure, ecological function (supporting services), and ES in those deep-sea settings subject to mining impact is a nascent but important topic of research for the future.

Acknowledgments

JTL received support from the Stout Foundation (20161390), the University of California San Diego's Frontiers of Innovation Scholars Program (g001) (which initiated collaboration with RTC), and the Program for Interdisciplinary Environmental Research (PIER) at the University of California San Diego and associated donors. LAL and JTL's contributions are inspired by the Deep-Ocean Stewardship Initiative and support from the JM Kaplan Fund (2014-333, 1579-20162011) for work on environmental issues associated with seabed mining. The authors are grateful to two anonymous reviewers and the editor for their insightful comments and suggestions.

References

26 U.S.C. § 9505. Harbor Maintenance Trust Fund.

Aburto-Oropeza, O., Ezcurra, E., Danemann, G., Valdez, V., Murray, J., Sala, E., 2008. Mangroves in the Gulf of California increase fishery yields. Proc. Natl. Acad. Sci. USA 105, 10456–10459. http://dx.doi.org/10.1073/pnas.0804601105.

Adams, P.B., 1980. Life history patterns in marine fishes and their consequences for fisheries management. Fish. Bull. 78, 1–12.

Althaus, F., Williams, A., Schlacher, T.A., Kloser, R.J., Green, M.A., Barker, B.A., Nax, N. J., Brodie, P., Schlacher-Hoenlinger, M.A., 2009. Impacts of bottom trawling on deep-coral ecosystems of seamounts are long-lasting. Mar. Ecol. Prog. Ser. 397, 279–294. http://dx.doi.org/10.3354/meps08248.

Armstrong, C.W., Foley, N.S., Tinch, R., van den Hove, S., 2012. Services from the deep: steps towards valuation of deep sea goods and services. Ecosyst. Serv. 2, 2–13. http://dx.doi.org/10.1016/j.ecoser.2012.07.001.

Auster, P.J., Moore, J., Heinonen, K.B., Watling, L., 2005. A habitat classification scheme for seamount landscapes: assessing the functional role of deep-water corals as fish habitat. Cold-water Corals Ecosyst., 761–769.

Auster, P.J., Gjerde, K., Heupel, E., Watling, L., Grehan, A., Rogers, A.D., 2011. Definition and detection of vulnerable marine ecosystems on the high seas: problems with the "move-on" rule. ICES J. Mar. Sci. 68, 254–264. http://dx.doi.org/10.1093/icesjms/fsq074.

Baillon, S., Hamel, J., Wareham, V.E., Mercier, A., 2012. Deep cold-water corals as nurseries for fish larvae. Front. Ecol. Environ. 10, 351–356. http://dx.doi.org/10.1890/120022.

Baker, B.J., Sheik, C.S., Taylor, C.A., Jain, S., Bhasi, A., Cavacoli, J.D., Dick, G.J., 2013. Community transcriptomic assembly reveals microbes that contribute to deep-sea carbon and nitrogen cycling. ISME J. 7, 1962–1973. http://dx.doi.org/10.11038/ismej.2013.85.

Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A., Byrnes, J., O'Connor, M.I., Hungate, B.A., Griffin, J.N., 2014. Linking biodiversity and ecosystem services: current uncertainties an the necessary next steps. Bioscience 64, 49–57. http://dx.doi.org/10.1093/biosci/bit003.

Barbier, E.B., Koch, E.W., Silliman, B.R., Hacker, S.D., Wolanski, E., Primavera, J., Granek, E.F., Polasky, S., Aswani, S., Cramer, L.A., Stoms, D.M., Kennedy, C.J., Bael, D., Kappel, C.V., Perillo, G.M.E., Reed, D.J., 2008. Coastal ecosystem-based management with nonlinear ecological functions and values. Science 319, 321. http://dx.doi.org/10.1126/science.1150349.

Barbier, E.B., Hacker, S.D., Kennedy, C., Kocj, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81, 169–193. http://dx.doi.org/10.1890/10-1510.1.

Batker, D., Schmidt, R., 2015. Environmental and Social Benchmarking Analysis of Nautilus Minerals Inc. Solwara 1 Project 88.

Baturin, G.N., 1971. Stages of phosphorite formation on the ocean floor. Nature 232, 61–62.

Beaumont, N.J., Austen, M.C., Mangi, S.C., Townsend, M., 2008. Economic valuation for the conservation of marine biodiversity. Mar. Pollut. Bull. 56, 386–396. http://dx.doi.org/10.1016/j.marpolbul.2007.11.013.

Bene, C., Phillips, M., Allison, E.H., 2011. The forgotten service: food as an ecosystem service from estuarine and coastal zones. In: Wolanski, E., McLusky, D.S. (Eds.), Treatise on Estuarine and Coastal Science, 12. Academic Press, Waltham, pp. 147–180.

Bennett, E.M., Cramer, W., Begossi, A., Cundill, G., Diaz, S., et al., 2015. Linking biodiversity, ecosystem services, and human well-being: three challenges for designing research for sustainability. Curr. Opin. Environ. Sustain. 14, 76–85. http://dx.doi.org/10.1016/j.cosust.2015.03.007.

Billings, A., Kaiser, C., Young, C.M., Hiebert, L.S., Cole, E., Wagner, J.K.S., Van Dover, C. L., 2016. SyPRID sampler: a large-volume, high-resolution, autonomous, deep-ocean precision plankton sampling system. Deep-Sea Res. II, In Press.

Blaustein, R.J., 2010. High-seas biodiversity and genetic resources: science and policy questions. BioScience 60, 408–413. http://dx.doi.org/10.1525/bio.2010.60.6.3.

Böhnke-Henrichs, A., Baulcomb, C., Koss, R., Hussain, S.S., de Groot, R.S., 2013. Typology and indicators of ecosystem services for marine spatial planning and management. J. Environ. Manag. 130, 135–145. http://dx.doi.org/10.1016/j.jenvman.2013.08.027.

Boschen, R.E., Rowden, A.A., Clark, M.R., Gardner, J.P.A., 2013. Mining of deep-sea seafloor massive sulfides: a review of the deposits, their benthic communities, impacts from mining, regulatory frameworks and management strategies. Ocean Coast. Manag. 84, 54–67. http://dx.doi.org/10.1016/j.ocecoaman.2013.07.005.

Boschen, R.E., Collins, P.C., Tunnicliffe, V., Carlsson, J., Gardner, J.P.A., Lowe, J., McCrone, A., Metaxas, A., Sinniger, F., Swaddling, A., 2016. A primer for use of genetic tools in selecting and testing the suitability of set-aside sites protected from deep-sea seafloor massive sulfide mining activities. Ocean Coast. Manag. 122, 37–48. http://dx.doi.org/10.1016/j.ocecoaman.2016.01.007.

Botsford, L.W., Brumbaugh, D.R., Grimes, C., Kellner, J.B., Largier, J., O'Farrell, M.R., Ralston, S., Soulanille, E., Wepestad, V., 2009. Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. Rev. Fish. Biol. Fish. 19, 69–95.

Boyd, J., Banzhaf, S., 2007. What are ecosystem services? The need for standardized environmental accounting units. Ecol. Econ. 63, 616–626. http://dx.doi.org/10.1016/j.ecolecon.2007.01.002.

Boyd, J., Ringold, P., Krupnick, A., Johnston, R.J., Weber, M.A., Hall, K., 2014. Ecosystem services indicators: improving the linkage between biophysical and economic analyses. Int. Rev. Environ. Resour. Econ. 8, 359–443. http://dx.doi.org/10.1561/101.00000073.

Brandt, A., 2005. Diversity of peracarid crustaceans (Malacostraca) from the abyssal plain of the Angola Basin. Org. Divers. Evol. 5, 105–112. http://dx.doi.org/10.1016/j.ode.2004.10.007.

Breithaupt, J.L., Smoak, J.M., Smith III, T.J., Sanders, C.J., Hoare, A., 2012. Organic carbon burial rates in mangrove sediments: strengthening the global budget. Glob. Biogeochem. Cycles 26, GB3011. http://dx.doi.org/10.1029/2012GB004375.

Bremner, J., Rogers, S.I., Frid, C.L.J., 2006. Matching biological traits to environmental conditions in marine benthic ecosystems. J. Mar. Syst. 60, 302. http://dx.doi.org/10.1016/j.jmarsys.2006.02.004.

Brummer, F., Pfannkuchen, M., Baltz, A., Hauser, T., Thiel, V., 2008. Light inside sponges. J. Exp. Mar. Biol. Ecol. 367, 61–64. http://dx.doi.org/10.1016/j.jembe.2008.06.036.

Buhl-Mortensen, L., Ellingsen, K.E., Buhl-Mortensen, P., Skaar, K.L., Gonzalez-Mireles, G., **73**, 2015. Trawling disturbance on megabenthos and sediment in the Barents Sea: chronic effects on density, diversity, and composition. ICES J. Mar. Sci . http://dx.doi.org/10.1093/icesjms/fsv200.

Burkhard, B., Kroll, F., Nedkov, S., Müller, F., 2012. Mapping ecosystem service supply, demand and budgets. Ecol. Indic. 21, 17–29. http://dx.doi.org/10.1016/j.ecolind.2011.06.019.

Campbell, L.M., Smith, C., 2006. What makes them pay? Values of volunteer tourists working for sea turtle conservation. Environ. Manag. 38, 84–96. http://dx.doi.org/10.1007/s00267-005-0188-0.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. http://dx.doi.org/10.1038/nature11148.

Carson, R.T., Flores, N.E., Mitchell, R.C., 1999. The theory and measurement of passive-use value. In: Bateman, I.J., Turner, K. (Eds.), Valuing Environmental Preferences: Theory and Practice of the Contingent Valuation Method in the US, EU, and Developing Countries. Oxford University Press, pp. 97–130.

Cathalot, C., Van Oevelen, D., Cox, T.J.S., Kutti, T., Lavaleye, M., Duineveld, G., Meysman, F.J.R., 2015. Cold-water coral reefs and adjacent sponge grounds: hotspots of benthic respiration and organic carbon cycling in the deep sea. Front. Mar. Sci. 2, 1–12. http://dx.doi.org/10.3389/fmars.2015.00037.

Chan, K.M.A., Shaw, M.R., Cameron, D.R., Underwood, E.C., Daily, G.C., 2006. Conservation planning for ecosystem services. PLoS Biol. 4, e379. http://dx.doi.org/10.1371/journal.pbio.0040379.

Chazdon, R.L., 2008. Beyond deforestation: restoring forests and ecosystem services on degraded lands. Science 320, 1458–1460. http://dx.doi.org/10.1126/science.1155365.

Chmura, G.L., Anisfeld, S.C., Cahoon, D.R., Lynch, J.C., 2003. Global carbon sequestration in tidal, saline wetland soils. Glob. Biogeochem. Cycles 17, 1111. http://dx.doi.org/10.1029/2002GB001917.

Clark, M.R., Rowden, A.A., Schlacher, T., Williams, A., Consalvey, M., Stocks, K.I., Rogers, A.D., O'Hara, T.D., White, M., Shank, T.M., Hall-Spencer, J.M., 2010. The ecology of seamounts: structure, function, and human impacts. Ann. Rev. Mar. Sci. 2, 253–278. http://dx.doi.org/10.1146/annurev-marine-120308-081109.

Collins, P.C., Croot, P., Carlsson, J., Colaço, A., Grehan, A., Hyeong, K., Kennedy, R., Mohn, C., Smith, S., Yamamoto, H., Rowden, A., 2013. A primer for the Environmental Impact Assessment of mining at seafloor massive sulfide deposits. Mar. Policy 42, 198–209. http://dx.doi.org/10.1016/j.marpol.2013.01.020.

Constable, A.J., 1999. Ecology of benthic macro-invertebrates in soft-sediment environments: a review of progress towards quantitative models and predictions. Aust. J. Ecol. 24, 452–476.

Continental Shelf Associates, Inc., 2006. Effects of oil and gas exploration and development on selected continental slope sites in the Gulf of Mexico, Volume I: Executive Summary. U.S. Department of the Interior, Minerals Management Service, New Orleans, LA. OCS Study MMS 2006-004. 45pp.

Convention on Biological Diversity, 2008. Decision adopted by the conference of the parties to the convention on biological diversity at its ninth meeting, IX/20 Marine and coastal biodiversity.

Council on Environmental Quality, 2005. Regulations for implementing the procedural provisions of the National Environmental Policy Act. Washington DC: Report 40 CFR Parts 1500-1517.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems, 2008. Ecol. Lett. 11, 1304–1315. http://dx.doi.org/10.1111/j.1461-0248.2008.01253.x.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Vincx, M., Gooday, A.J., 2008. Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Curr. Biol. 18, 1–8. http://dx.doi.org/10.1016/j.cub.2007.11.056.

de Groot, R., Fisher, B., Christie, M., Aronson, J., Braat, L., Gowdy, J., Haines-Young, R., Maltby, E., Neuville, A., Polasky, S., Portela, R., Ring, I., 2010. Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation. In: Kumar, P. (Ed.), The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. Earthscan, London and Washington.

Dell'Anno, A., Carugati, L., Corinaldesi, C., Riccioni, G., Danovaro, R., 2015. Unveiling the biodiversity of deep-sea nematodes through metabarcoding: are we ready to bypass the classical taxonomy? PLoS One 10, e0144928. http://dx.doi.org/10.113711/journal.pone.0144928.

Devine, J.A., Baker, K.D., Haedrich, R.L., 2006. Fisheries: deep-sea fishes qualify as endangered. Nature 439, 29. http://dx.doi.org/10.1038/439029a.

Dubilier, N., Bergin, C., Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat. Rev. Microbiol. 6, 725–740. http://dx.doi.org/10.1038/nrmicro1992.

Ehrlich, H., Etnoyer, P., Litvinov, S.D., Olennikova, M.M., Domaschke, H., Hanke, T., Born, R., Meissner, H., Worch, H., 2006. Biomaterial structure in deep-sea bamboo coral (Anthozoa: Gorgonacea: Isididae): perspectives for the development of bone implants and templates for tissue engineering. Mater. Sci. Eng. Technol. 37, 552–557. http://dx.doi.org/10.1002/mawe.200600036.

Erwin, P.M., López-legentil, S., Schuhmann, P.W., 2010. The pharmaceutical value of marine biodiversity for anti-cancer drug discovery. Ecol. Econ. 70, 445–451. http://dx.doi.org/10.1016/j.ecolecon.2010.09.030.

Farber, S.C., Costanza, R., Wilson, M.A., 2002. Economic and ecological concepts for valuing ecosystem services. Ecol. Econ. 41, 375–392. http://dx.doi.org/10.1016/S0921-8009(02)00088-5.

Feely, R.A., Sabine, C.L., Takahashi, T., Wanninkhof, R., 2001. Uptake and storage of carbon dioxide in the ocean: the glocal CO2 survey. Oceanography 14, 18–32.

Findlay, K.P., Best, P.B., Ross, G.J.B., Cockcroft, V.G., 1992. The distribution of small odontocete cetaceans off the coasts of South Africa and Namibia. S. Afr. J. Mar. Sci. 12, 237–270. http://dx.doi.org/10.2989/02577619209504706.

Fisher, B., Turner, R.K., Morling, P., 2009. Defining and classifying ecosystem services for decision making. Ecol. Econ. 68, 643–653. http://dx.doi.org/10.1016/j.ecolecon.2008.09.014.

Fisher, C.R., Hsing, P., Kaiser, C.L., Yoerger, D.R., Roberts, H.H., Shedd, W.W., Cordes, E.E., Shank, T.M., Berlet, S.P., Saunders, M.G., Larcom, E.A., Brooks, J.M., 2014. Footprint of Deepwater Horizon blowout impact to deep-water coral communities. Proc. Natl. Acad. Sci. USA 111, 11744–11749. http://dx.doi.org/10.1073/pnas.1403492111.

Flores, J.F., Fisher, C.R., Carney, S.L., Green, B.N., Freytag, J.K., Schaeffer, S.W., Royer Jr., W.E., 2005. Sulfide binding is mediated by zinc ions discovered in the crystal structure of a hydrothermal vent tubeworm hemoglobin. Proc. Natl. Acad. Sci. USA 102, 2713–2718. http://dx.doi.org/10.1073/pnas.0407455102.

Foley, N.S., Rensburg, T.M., Van, Armstrong, C.W., 2011. The rise and fall of the Irish orange roughy fishery: an economic analysis. Mar. Policy 35, 756–763. http://dx.doi.org/10.1016/j.marpol.2011.01.003.

Föllmi, K.B., 1996. The phosphorous cycle, phosphosgenesis and marine phosphate-rich deposits. Earth-Sci. Rev. 40, 55–124. http://dx.doi.org/10.106/0012-8252(95)00049-6.

Food and Agriculture Organization of the United Nations, 2009a. Report of the technical consultation on international guidelines for the management of deep-sea fisheries in the high seas. FAO Fisheries and Aquaculture Report.

Food and Agriculture Organization of the United Nations, 2009b. Report of the technical consultation on international guidelines for the management of deep-sea fisheries in the high seas: Annex. FAO Fisheries and Aquaculture Report.

Frid, C.L.J., Paramor, O.A.L., Brockington, S., Bremner, J., 2008. Incorporating ecological functioning in to the designation and management of marine protected areas. Hydrobiologia 606, 69–79. http://dx.doi.org/10.1007/s10750-008-9343-y.

Gedan, K.B., Kirwan, M.L., Wolanski, E., Barbier, E.B., Silliman, B.R., 2011. The present and future role of coastal wetland vegetation in protecting shorelines: answering recent challenges to the paradigm.

Gibson, J.F., Stein, E.D., Baird, D.J., Finlayson, C.M., Zhang, X., Hajibabaei, 2015. Wetland ecogenomics – The next generation of wetland biodiversity and functional assessment. Wetl. Sci. Pract. 32, 27–32.

Glenn, H., Wattage, P., Mardle, S., Rensburg, T., Van, Grehan, A., Foley, N., 2010. Marine protected areas — substantiating their worth. Mar. Policy 34, 421–430. http://dx.doi.org/10.1016/j.marpol.2009.09.007.

Glover, A., Paterson, G., Bett, B.J., Gage, J., Sibuet, Myriam, Sheader, M., Hawkins, L., 2001. Patterns in polychaete abundance and diversity from the Madeira Abyssal Plain, northeast Atlantic. Deep. Res. Part I 48, 217–236. http://dx.doi.org/10.1016/S0967-0637(00)00053-4.

Glover, A.G., Smith, C.R., 2003. The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. Environ. Conserv. 30. http://dx.doi.org/10.1017/S0376892903000225.

Glover, A., Dahlgren, T., Wiklund, H., Mohrbeck, I., Smith, C., 2015. An End-to-End DNA taxonomy methodology for benthic biodiversity survey in the Clarion-Clipperton Zone, Central Pacific Abyss. J. Mar. Sci. Eng. 4, 2. http://dx.doi.org/10.3390/jmse4010002.

Glover, A.G., Wiklund, H., Rabone, M., Amon, D.J., Smith, C.R., O'Hara, T., Mah, C.L., Dahlgren, T.G., 2016. Abyssal fauna of the UK-1 polymetallic nodule exploration claim, Clarion-Clipperton Zone, central Pacific Ocean: Echinodermata. Biodivers. Data J. 4, e7251. http://dx.doi.org/10.3897/BDJ.4.e7251.

Godet, L., Zelnio, K.A., Van, Dover C.L., 2011. Scientists as stakeholders in conservation of hydrothermal vents. Conserv. Biol. 25, 214–222. http://dx.doi.org/10.111/j.1523-1739.2010.01642.

Golder Associates, 2012. Mineral resource estimate: Solwara Project, Bismarck Sea, PNG. Report number: 107631040-003-R-Rev 1.

Govenar, B., 2010. Shaping vent and seep communities: habitat provision and modification by foundation species, in: Kiel, S., (ed.), The Vent and Seep Biota: Aspects from Microbes to Ecosystems.

Groffman, P.M., Baron, J.S., Blett, T., Gold, A.J., Goodman, I., Gunderson, L.H., Levinson, B.M., Palmer, M.A., Paerl, H.W., Peterson, G.D., Poff, N.L., Rejeski, D.W., Reynolds, J.F., Turner, M.G., Weathers, K.C., Wiens, J., 2006. Ecological thresholds: the key to successful environmental management or an important concept with no practical application? Ecosystems 9, 1–13. http://dx.doi.org/10.1007/s10021-003-0142-z.

Gunderson, L.H., Cosnes, B., Garmestani, A.S., 2016. Adaptive governance of riverine and wetland ecosystem goods and services. J. Environ. Manag . http://dx.doi.org/10.1016/j.jenvman.2016.05.024.

Haines-Young, R., Potschin, M., 2013. Common International Classification of Ecosystem Services (CICES): Consultation on Version 4, August-December 2012. EEA Framework Contract No EEA/IEA/09/003.

Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C.D., Bruno, J.F., Casey, K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E.M.P., Perry, M.T., Selig, E.R., Spalding, M., Steneck, R., Watson, R., 2008. A global map of human impact on marine ecosystems. Science 319, 948–952. http://dx.doi.org/10.1126/science.1149345.

Hanley, N., Barbier, E.B., 2009. Pricing Nature: Cost-Benefit Analysis and Environmental Policy. Edward Elgar; Cheltenham, UK and Northampton, USA.

Harrison, P.A., Berry, P.M., Simpson, G., Haslett, J.R., Blicharska, M., Bucur, M., Dunford, R., Egoh, B., Garcia-Llorente, M., Geamana, N., Geertsema, W., Lommelen, E., Meiresonne, L., Turkelboom, F., 2014. Linkages between biodiversity attributes and ecosystem services: a systematic review. Ecosyst. Serv. 9, 191–203. http://dx.doi.org/10.1016/j.ecoser.2014.05.006.

Hartnett, H.E., Keil, R.G., Hedges, J.I., Devol, A.H., 1998. Influence of osygen exposure time on organic carbon preservation in continental margin sediments. Nature 391, 572–574.

Hein, J.R., Koschinsky, A., Bau, M., Manheim, F.T., Kang, J.K., 2000. Cobalt-Rich Ferromanganese Crusts in the Pacific. In: Cronan, D.S. (Ed.), Handbook of Marine Mineral Deposits. CRC Press; Boca Raton, USA.

Hein, J.R., Mizell, K., Koschinsky, A., Conrad, T.A., 2013. Deep-ocean mineral deposits as a srouce of critical metals for high- and green- technology applications: comparison with land0based resources. Ore Geol. Rev. 51, 1–14. http://dx.doi.org/10.1016/j.oregeorev.2012.12.001.

Higgs, N.D., Attrill, M.J., 2015. Biases in biodiversity: wide-ranging species are discovered first in the deep sea. Front. Mar. Sci. 2, 61. http://dx.doi.org/10.3389/fmars.2015.00061.

Hoff, G.R., Stevens, B., 2005. Faunal assemblage structure on the Patton Seamount (Gulf of Alaska, USA). Alsk. Fish. Res. Bull. 11, 27–36.

Hopkinson, C.S., Cai, W.J., Hu, X., 2012. Carbon sequestration in wetland dominated coastal systems-a global sink of rapidly diminishing magnitude. Curr. Opin. Environ. Sustain. 4, 186–194. http://dx.doi.org/10.1016/j.cosust.2012.03.005.

Howell, K.L., Billett, D.S.M., Tyler, P.A., 2002. Depth-related distribution and abundance of seastars (Echinodermata: Asteroidea) in the Porcupine Seabight and Porcupine Abyssal Plain, N.E. Atlantic. Deep Res. Part I 49, 1901–1920. http://dx.doi.org/10.1016/S0967-0637(02)00090-0.

Hughes, S.E., 1975. Initial U.S. Exploration of Nine Gulf of Alaska Seamounts and Their Associated Fish and Shellfish Resources. Northwest and Alaska Fisheries Center, NOAA.

International Seabed Authority, 2010a. Decision of the Assembly of the International Seabed Authority Relating to the Regulations on Prospecting and Exploration for Polymetallic Sulfides in the Area. International Seabed Authority, Kingston, Jamaica, ISBA/16/A/12/Rev.1.

International Seabed Authority, 2010b. Decision of the Assembly of the International Seabed Authority Relating to the Regulations on Prospecting and Exploration for Cobalt-rich Ferromanganese Crusts in the Area. International Seabed Authority, Kingston, Jamaica, ISBA/18/A/11.

International Seabed Authority, 2010c. Decision of the Assembly of the International Seabed Authority Relating to the Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area. International Seabed Authority, Kingston, Jamaica, ISBA/19/A/9.

International Seabed Authority, 2011. Environmental Management Plan for the Clarion-Clipperton Zone. IBSA/17/LTC/7. Kingston, Jamaica.

International Seabed Authority, 2013a. Recommendations for the Guidance of Contractors for the Assessment of the Possible Environmental Impacts Arising from Exploration for Marine Minerals in the Area. International Seabed Authority, Kingston, Jamaica, IBSA/19/LTC/8.

International Seabed Authority, 2013b. Decision of the Council of the International Seabed Authority relating to amendments to the Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area and related matters. IBSA/19/C/17. Kingston, Jamaica.

International Seabed Authority, 2015. Developing a Regulatory Framework for Mineral Exploitation in the Area. International Seabed Authority, Kingston, Jamaica.

International Seabed Authority, 2016. Working Draft Regulations and Standard Contract Terms on Exploitation for Mineral Resources in the Area. International Seabed Authority, Kingston, Jamaica.

Jaeckel, A., Ardron, J.A., Gjerde, K.M., **70**, 2016a. Sharing benefits of the common heritage of mankind – Is the deep seabed mining regime ready? Mar. Policy . http://dx.doi.org/10.1016/j.marpol.2016.03.009.

Jaeckel, A., **70**, 2016b. Deep seabed mining and adaptive management: the procedural challenges for the International Seabed Authority. Mar. Policy . http://dx.doi.org/10.1016/j.marpol.2016.03.008.

Jahnke, R.A., 1990. Early diagenesis and recycling of biogenic debris at the seafloor, Santa Monica Basin, California. J. Mar. Res. 48, 413–436.

Janssen, A., Kaiser, S., Meißner, K., Brenke, N., Menot, L., **10**, 2015. A reverse taxonomic approach to assess macrofaunal distribution patterns in abyssal pacific polymetallic nodule fields. PLos One . http://dx.doi.org/10.1371/journal.pone.0117790.

Jeanthon, C., 2000. Molecular Ecology of Hydrothermal Vent Microbial Communities. Kluwer Academic Publishers, The Netherlands.

Jobstvogt, N., Townsend, N., Witte, U., Hanley, N., 2014a. How can we identify and communication the ecological value of deep-sea ecosystem services? PLoS One 9 (7), e100646. http://dx.doi.org/10.1371/journal.pone.0100646.

Jobstvogt, N., Hanley, N., Hynes, S., Kenter, J., Witte, U., 2014b. Twenty thousand sterling under the sea: estimating the value of protecting deep-sea biodiversity. Ecol. Econ. 97, 10–19. http://dx.doi.org/10.1016/j.ecolecon.2013.10.019.

Jones, J.B., 1992. Environmental impact of trawling on the seabed: a review. New Zealand J. Mar. Freshw. Res. 26, 59–67. http://dx.doi.org/10.10880/00288330.1992.9516500.

Jørgensen, B.B., Boetius, A., **5**, 2007. Feast and famine — microbial life in the deep-sea bed. Nat. Rev . http://dx.doi.org/10.1038/nrmicro1745.
Koch, E.W., Barbier, E.B., Silliman, B.R., Reed, D.J., Perillo, G.M.E., Hacker, S.D., Granek, E.F., Primavera, J.H., Muthiga, N., Polasky, S., Halpern, B.S., Kennedy, C.J., Kappel, C.V., Wolanski, E., 2009. Non-linearity in ecosystem services: temporal and spatial variability in coastal protection. Front. Ecol. Environ. 7, 29–37. http://dx.doi.org/10.1890/080126.
Koslow, J.A., 1997. Seamounts and the Ecology of Deep-Sea Fisheries: the firm-bodied fishes that feed around seamounts are biologically distinct from their deepwater neighbors—and may be especially vulnerable to overfishing. Am. Sci. 85, 168–176. http://dx.doi.org/10.2307/27856735.
Kremen, C., 2005. Managing ecosystem services: what do we need to know about their ecology? Ecol. Lett. 8, 468–479. http://dx.doi.org/10.1111/j.1461-0248.2005.00751.x.
Kuypers, M.M.M., Pancost, R.D., Nijenhuis, I.A., Damste, J.S.S., 2002. Enhanced productivity lef to increased organic carbon burial in the euxinic North Atlantic basin during the late Cenomanian oceanic anoxic event. Paleoceanography 17, 1051. http://dx.doi.org/10.1029/2000PA000569.
Lallier, L., Maes, F., 2016. Environmental impact assessment procedure for deep seabed mining in the Area: independent expert review and public participation. Mar. Policy.
Landers, D.H., Nahlik, A.M., 2013. Final Ecosystem Goods and Services Classification System (FEGS-CS). U.S. Environmental Protection Agency, Washington D.C., EPA/600/R-13/ORD-004914.
Leary, D.K., **1**, 2004. Bioprospecting and the genetic resources of hydrothermal vents on the high seas: what is the existing legal position, where are we heading and what are our options? Macquarie J. Int. Comp. Environ. Law, 137.
Leduc, D., Rowden, A.A., Torres, L.G., Nodder, S.D., Pallentin, A., 2015. Distribution of macro-faunal communities in phosphorite nodule deposits on Chatham Rise, Southwest Pacific: implications for management of seabed mining. Deep-Sea Res. Pt. I 99, 105–118. http://dx.doi.org/10.10016/j.dsr.2015.01.006.
Levin, L.A., Le Bris, N., 2015. The deep ocean under climate change. Science 350, 766–768. http://dx.doi.org/10.1126/science.aad0126.
Levin, L.A., Baco, A.R., Bowden, D., Calaco, A., Cordes, E.E., Cunha, M.R., Demopoulos, A., Gobin, J., Grupe, B., Le, J., Metaxas, A., Netburn, A., Rouse, G.W., Thurber, A.R., Tunnicliffe, V., Van Dover, C.L., Vanreusel, A., Watling, L., 2016. Hydrothermal vents and methane seeps: rethinking the sphere of influence. Front. Mar. Sci. 3. http://dx.doi.org/10.3389/fmars.2016.00072.
Lévy, J.P., 2014. The International Seabed Authority: 20 Years. The International Seabed Authority, Jamaica.
Lim, K.H., Grebitus, C., Hu, W., Nagaya Jr., R.M., 2015. More than meets the eye: Consumers' willingness to pay for Marine Stewardship Council's certified seafood. Selected paper prepared for presentation at the 2015 Agricultural & Applied Economics Association and Western Agriculture Economics Association Annual Meeting, San Francisco, CA, July 26–28.
Lin, Q., Mendelssohn, I.A., 2012. Impacts and recovery of the deepwater horizon oil spill on vegetation structure and function of coastal salt marshes in the Northern Gulf of Mexico. Environ. Sci. Technol. 46, 3737–3743.
Liquete, C., Piroddi, C., Drakou, E.G., Gurney, L., Katsanevakis, S., Charef, A., Egoh, B., 2013. Current status and future prospects for the assessment of marine and coastal ecosystem services: a systematic review. PLoS One 8 (7), e67737. http://dx.doi.org/10.1371/journal.pone.0067737.
Maes, J., Egoh, B., Willemen, L., Liquete, C., Vihervaara, P., Schagner, J.P., Grizzetti, B., et al., 2012. Mapping ecosystem services for policy support and decision making in the European Union. Ecosyst. Serv. 1, 31–39. http://dx.doi.org/10.1016/j.ecoser.2012.06.004.
Mahon, B.P., Bhatt, A., Vulla, D., Supuran, C.T., McKenna, R., 2015. Exploration of anionic inhibition of the α-carbonic anhydrase from Thiomicrospira crunogena XCL-2 gammaproteobacterium: a potential bio-catalytic agent for industrial CO2 removal. Chem. Eng. Sci. 138, 575–580. http://dx.doi.org/10.1016/j.ces.2015.07.030.
Majone, G., 2002. What price safety? The precautionary principle and its policy implications. J. Common Mark. Stud. 40, 89–109.
Mangi, S.C., Davis, C.E., Payne, L.A., Austen, M.C., Simmonds, D., Beaumont, N.J., Smyth, T., 2011. Valuing the regulatory services provided by marine ecosystems. Environmetrics 22, 686–698.
Martín, J., Puig, P., Palanques, A., Ribó, M., 2014. Trawling-induced daily sediment resuspension in the flank of a Mediterranean submarine canyon. Deep Sea Res. II 104, 174–183. http://dx.doi.org/10.1016/j.dsr2.2013.05.036.
Martin, S.L., Balance, L.T., Groves, T., 2016. An ecosystem services perspective for the oceanic Eastern Tropical Pacific: commercial fisheries, carbon storage, recreational fishing, and biodiversity. Front. Mar. Sci. 3, 50. http://dx.doi.org/10.3389/fmars.2016.00050.
McClain, C.R., Barry, J.P., 2010. Habitat heterogeneity, disturbance, and productivity work in concert to regulate biodiversity in deep submarine canyons. Ecology 91, 964–976.
Mcleod, E., Chmura, G.L., Bouillon, S., Salm, R., Björk, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., Silliman, B.R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO 2. Front. Ecol. Environ. 9, 552–560. http://dx.doi.org/10.1890/110004.
McMurtry, G., 2001. Authigenic deposits In: S.A. Thorpe K.K. Turekin (Eds.), Encyclopedia of Ocean Sciences, London.
Mengerink, K.J., Dover, C.L. Van, Ardron, J., Baker, M., Escobar-briones, E., Gjerde, K., Koslow, J.A., Ramirez-llodra, E., Lara-lopez, A., Squires, D., Sutton, T., Sweetman, A.K., Levin, L.A., 2014. A call for deep-ocean stewardship. Science. 334, 696–698.
Merrie, A., Dunn, D.C., Metian, M., Boustany, A.M., Takei, Y., Elferink, A.O., Ota, Y., Christensen, V., Halpin, P.N., Österblom, H., 2014. An ocean of surprises – Trends in human use, unexpected dynamics and governance challenges in areas beyond national jurisdiction. Glob. Environ. Change 27, 19–31. http://dx.doi.org/10.1016/j.gloenvcha.2014.04.012.
Meyer-Lombard, D.R., Amend, J.P., Osburn, M.R., 2013. Microbial diversity and potential for arsenic and iron biogeochmical cycling at an arsenic rich, shallow-sea hydrothermal vent (Tutum Bay, Papua New Guinea). Chem. Geol. 348, 37–47.
Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington DC.
Mitchell, R.C., Carson, R.T., 1989. Using Surveys to Value Public Goods: The Contingent Valuation Method. Resources for the Future, Washington D.C.
Mitwally, H.M., Fleeger, J.W., 2016. A test of biological trait analysis with nematodes and an anthropogenic stressor. Environ. Monit. Assess. 188, 140. http://dx.doi.org/10.1007/s10661-016-5128-3.
Morato, T., Watson, R., Pitcher, T.J., Pauly, D., 2006. Fishing down the deep. Fish. Fish. 7, 24–34. http://dx.doi.org/10.1111/j.1467-2979.2006.00205.x.
Morato, T., Hoyle, S.D., Allain, V., Nicol, S.J., 2010. Seamounts are hotspots of pelagic biodiversity in the open ocean. Proc. Natl. Acad. Sci. USA 107, 9707–9711. http://dx.doi.org/10.1073/pnas.0910290107.
Morgan, C.L., 2000. Resource estimates of the Clarion-Clipperton Fracture Zone manganese nodule deposits. In: Cronan, D.S. (Ed.), Handbook of Marine Mineral Deposits. CRC Press; Baton Roca, USA.
Morgan, N.B., Cairns, S., Reiswig, H., Baco, A.R., 2015. Benthic megafaunal community structure of cobalt-rich manganese crusts on Necker Ridge. Deep-Sea Res. I 104, 92–105.
Mullineaux, L.S., 1987. Organisms living on manganese nodules and crusts: distribution and abundance at three North Pacific sites. Deep-Sea Res. 34, 165–184.
Mumby, P.J., Chollet, I., Bozec, Y.M., Wolff, N.H., 2014. Ecological resilience, robustness, and vulnerability: how do these concepts benefit ecosystem management? Curr. Opin. Environ. Sustain. 7, 22–27. http://dx.doi.org/10.1016/j.cosust.2013.11.021.
Naidoo, R., Balmford, A., Costanza, R., Fisher, B., Green, R.E., Lehner, B., Malcolm, T.R., Ricketts, T.H., 2008. Global mapping of ecosystem services and conservation priorities. Proc. Natl. Acad. Sci. USA 105, 9495–9500.
Nakajima, R., Yamamoto, H., Kawagucci, S., Takaya, Y., Nozaki, T., Chen, C., Fujikura, K., Miwa, T., Takai, K., 2015. Post-drilling changes in seabed landscape and megabenthos in a deep-sea hydrothermal system, the Iheya North Field, Okinawa Trough. PLoS One 10, e0123095. http://dx.doi.org/10.1371/journal.pone.0123095.
Nautilus Minerals Niugini Limited, 2008. Environmental Impact Statement: Solwara I Project, Executive Summary. Coffey Natural Systems, Brisbane.
Norse, E.A., Brooke, S., Cheung, W.W.L., Clark, M.R., Ekeland, I., Froese, R., Gjerde, K.M., Haedrich, R.L., Heppell, S.S., Morato, T., Morgan, L.E., Pauly, D., Sumaila, R., Watson, R., 2012. Sustainability of deep-sea fisheries. Mar. Policy 36, 307–320.
Oberle, F.K.J., Storlazzi, C.D., Hanebuth, T.J.J., 2016. What a drag: quantifying the global impact of chronic bottom trawling on continental shelf sediment. J. Mar. Syst. 159, 109–119. http://dx.doi.org/10.1016/j.jmarsys.2015.12.007.
O'Farrell, P.J., De Lange, W.J., Le Maitre, D.C., Reyers, B., Blignaut, J.N., 2011. The possibilities and pitfalls presented by a pragmatic approach to ecosystem service valuation in an arid biodiversity hotspot. J. Arid. Environ. 75, 612–623. http://dx.doi.org/10.1016/j.jaridenv.2011.01.005.
O'Garra, T., 2009. Bequest values for marine resources: how important for indigenous communities in less-developed economies? Environ. Resour. Econ. 44, 179–202. http://dx.doi.org/10.1007/s10640-009-9279-3.
Oebius, H.U., Becker, H.J., Rolinski, S., Jankowski, J.A., 2001. Parametrization and evaluation of marine environmental impacts produced by deep-sea manganese nodule mining. Deep-Sea Res. II 48, 3453–3467.
Palumbi, S.R., Sandifer, P.A., Allan, J.D., Beck, M.W., Fautin, D.G., Fogarty, M.J., Halpern, B.S., Incze, L.S., Leong, J.A., Norse, E., Stachowicz, J.J., Wall, D.H., 2009. Managing for ocean biodiversity to sustain marine ecosystem services. Front. Ecol. Environ. 7, 204–211. http://dx.doi.org/10.1890/070135.
Partidario, M.R., Gomes, R.C., 2013. Ecosystem services inclusive strategic environmental assessment. Environ. Impact Assess. Rev. 40, 36–46. http://dx.doi.org/10.1016/j.eiar.2013.01.001.
Petersen, S., Krätschell, A., Augustin, N., Jamieson, J., Hein, J.R. and Hannington, M.D., News from the seabed – geological characteristics and resource potential of deep-sea mineral resources. Mar. Policy **70**, 2016, 175–187, http://dx.doi.org/10.1016/j.marpol.2016.03.012.
Puig, P., Canals, M., Company, J.B., Martin, J., Amblas, D., Lastras, G., Palanques, A., Calafat, A.M., 2012. Ploughing the deep sea floor. Nature 489, 286–290. http://dx.doi.org/10.1038/nature11410.
Pusceddu, A., Bianchelli, S., Martín, J., Puig, P., Palaneques, A., Masqué, P., Danovaro, R., 2014. Chronic and intensive bottom trawling impairs deep-sea biodiversity and ecosystem functioning. Proc. Natl. Acad. Sci. USA 111, 8861–8866. http://dx.doi.org/10.1073/pnas.1405454111.
Ramirez-Llodra, E., Shank, T.M., German, C.R., 2007. Biodiversity and biogeography of hydrothermal vent species. Oceanography 20, 30–41.
Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., Vecchione, M., 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences 7, 2851–2899. http://dx.doi.org/10.5194/bg-7-2851-2010.

Ramirez-Llodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A., Menot, L., Rowden, A.A., Smith, C.R., Van Dover, C.L., **6**, 2011. Man and the last great wilderness: human impact on the deep sea. PLoS One, e22588. http://dx.doi.org/10.1371/journal.pone.0022588.

Raudsepp-Hearne, C., Peterson, G.D., Bennett, E.M., 2010. Ecosysterm service bundles for analyzing tradeoffs in diverse landscapes. Proc. Natl. Acad. Sci. USA 107, 5242–5247. http://dx.doi.org/10.1073/pnas.0907284107.

Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L., Nelson, R.K., Carmichael, C.A., Mcintyre, C.P., Fenwick, J., Ventura, G.T., 2012. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc. Natl. Acad. Sci. USA 109, 20229–20234. http://dx.doi.org/10.1073/pnas.1101242108.

Reeves, R.R., 2000. The Value of Sanctuaries, Parks, and Reserves (Protected Areas) as Tools for Conserving Marine Mammals. Marine Mammal Commission, Bethesda, MD.

Reise, K., 2002. Sediment mediated species interactions in coastal waters. J. Sea Res. 48, 127–141.

Resing, J.A., Sedwick, P.N., German, C.R., Jenkins, W.J., Moffett, J.W., Sohst, B.M., Tagliabue, A., 2015. Basin-scale transport of hydrothermal dissolved metals across the South Pacific Ocean. Nature 523, 200–203. http://dx.doi.org/10.1038/nature14577.

Rex, M.A., McClain, C.R., Johnson, N.A., Etter, R.J., Allen, J.A., Bouchet, P., Warén, A., 2005. A source-sink hypothesis for Abyssal biodiversity. Am. Nat. 165, 163–178. http://dx.doi.org/10.1086/427226.

Rex, M.A., Etter, R.J., 2010. Deep-Sea Biodiversity: Pattern and Scale. Harvard University Press; Cambridge, USA and London, UK.

Rio Declaration on Environment and Development, 1992. Principle 15.

Roberts, C.M., 2002. Deep impact: the rising toll of fishing in the deep sea. Trends Ecol. Evol. 17, 242–245. http://dx.doi.org/10.1016/S0169-5347(02)02492-8.

Rogers, A., Harborne, A.R., Brown, C.J., Bozec, Y.M., Castro, C., et al., 2015. Anticipative management for coral reef ecosystem services in the 21st century. Glob. Change Biol. 21, 504–514. http://dx.doi.org/10.111/gcb.12725.

Rosenbaum, H., Grey, F., 2015. A critique of the Nautilus Minerals environmental and social benchmarking analysis of the Solwara I project. Deep Sea Mining Campaign.

Ruan, L., Bian, X., Wang, X., Yan, X., Li, F., Xu, X., 2008. Molecular characteristics of the tubeworm, *Ridgeia piscesae*, from the deep-sea hydrothermal vent. Extremophiles 12, 735–739. http://dx.doi.org/10.1007/s00792-008-0172-8.

Schlacher, T.A., Baco, A.R., Rowden, A.A., O'Hara, T.D., Clark, M.R., Kelley, C., Dower, J. F., 2014. Seamount benthos in a cobalt-rich crust region of the central Pacific: conservation challenges for future seabed mining. Divers. Distrib. 20, 491–502. http://dx.doi.org/10.1111/ddi.12142.

Schmidt, C.W., 2015. Going deep: cautious steps toward seabed mining. Environ. Health Perspect. 123, A234–A241. http://dx.doi.org/10.1289/ehp.123-A234.

Schriever, C., Ahnert, A., Bluhm, H., Browoski, C., Thiel, H., 1997. Results of the Large Scale Deep-sea Environmental Impact Study DISCOL During Eight Years of Investigation. International Society of Offshore and Polar Engineers; Honolulu, USA.

Schulz, H.N., Schulz, H.D., 2005. Large sulfur bacteria and the formation of phosphorite. Science 307, 416–418. http://dx.doi.org/10.1126/science.1103096.

Secretariat of the Pacific Community, 2012. Pacific-ACP States Regional Legislative and Regulatory Framework for Deep Sea Minerals Exploration and Exploitation. SPC SOPAC Division Published Report 111. Suva, Fiji.

Seidl, R., Spies, T.A., Peterson, D.L., Stephens, S.L., Hicke, J.A., 2016. Searching for resilience: addressing the impacts of changing disturbance regimes on forest ecosystem services. J. Appl. Ecol. 53, 120–129. http://dx.doi.org/10.1111/1365-2664.12511.

Seymour, J.F., **31**, 2004. Hardrock mining and the environment: issues of federal enforcement and liability. Ecol. Law Q., 795.

Shimmield, T.M., Black, K.D., Howe, J.A., Hughes, D.J., Sherwin, T., 2010. Final Report: Independent Evaluation of Deep-sea Mine Tailings Placement (DSTP) in PNG.. SAMS Research Services Limited, Project number: 8.ACP.PNG.18-B/1.

Shuter, B.J., Jones, M.L., Korver, R.M., Lester, N.P., 1998. A general, list history based model for regional management of fish stocks: the inland lake trout (*Salvelinus namaycush*) fisheries of Ontario. Can. J. Fish. Aquat. Sci. 55, 2161–2177.

Sinniger, F., Pawlowski, J., Harii, S., Gooday, A.J., Yamamoto, H., et al., 2016. Worlwide analysis of sedimentary DNA reveals major gaps in taxonomix knowledge of deep-sea benthos. Front. Mar. Sci. 3, 92. http://dx.doi.org/10.3389/fmars.2016.00092.

Skropeta, D., Wei, L., 2014. Recent advances in deep-sea natural products. Nat. Prod. Rep. 31, 999. http://dx.doi.org/10.1039/c3n70118b.

Smith, C.R., Levin, L.A., Koslow, A., Tyler, P.A., Glover, A.G., 2008. The near future of the deep seafloor ecosystem. In: Polunin, N. (Ed.), Aquatic Ecosystems. Cambridge University Press, Cambridge, pp. 334–351.

Smith, R.W., Bianchi, T.S., Allison, M., Savage, C., Galy, V., 2015. High rates of organic carbon burial in fjord sediments globally. Nat. Geosci. 8, 450–453. http://dx.doi.org/10.1038/NGEO02421.

Tagliabue, A., Bopp, L., Dutay, J., Bowie, A.R., Chever, F., Jean-Baptiste, P., Bucciarelli, E., Lannuzel, D., Remeny, T., Sarthou, G., Aumont, O., Gehlen, M., Jeandel, C., 2010. Hydrothermal contribution to the oceanic dissolved iron inventory. Nat. Geosci. 3, 252–256.

Terpe, K., 2013. Overview of thermostable DNA polymerases for classical PCR applications: from molecular and biochemical fundamentals to commercial systems. Appl. Microbiol. Biot. 97, 10243–10254.

Therivel, R., Partidario, M.R., 1996. The Practice of Strategic Environmental, Assessment. Earthscan Publications, New York.

Thiel, H., Schriever, G., Bussau, C., Borowski, C., 1993. Manganese nodule crevice fauna. . Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 419–423. http://dx.doi.org/10.1016/0967-0637(93)90012-R.

Thiel, H., Schriever, G., Ahnert, A., Bluhm, H., Borowski, C., Vopel, K., 2001. The large-scale environmental impact experiment DISCOL – reflection and foresight. Deep Sea Res. II 48, 3869–3882. http://dx.doi.org/10.1016/S0967-0645(01)00071-6.

Thurber, A.R., Sweetman, A.K., Narayanaswamy, B.E., Jones, D.O.B., Ingels, J., Hansman, R.L., 2014. Ecosystem function and services provided by the deep sea. Biogeosci. Discuss. 11, 3941–3963. http://dx.doi.org/10.5194/bgd-11-3941-2014.

Tunnicliffe, V., Embley, R.W., Holden, J.F., Butterfield, D.A., Massoth, G.J., Juniper, S. K., 1997. Biological colonization of new hydrothermal vents following an eruption on Juan de Fuca Ridge. Deep Sea Res. I 44, 1627–1644. http://dx.doi.org/10.1016/S0967-0637(97)00041-1.

United Nations Convention on the Law of the Sea, 1982. Article 136.

United Nations Convention on the Law of the Sea, 1982. Article 140.

United Nations, 2016. Chapter 55: Overall Value of the Oceans to Humans, in: World Ocean Assessment.

United States Fish and Wildlife, 2002. National Wetlands Mitigation Action Plan.

Usui, A., Graham, I.J., Ditchburn, R.G., Zondervan, A., Shibasaki, H., Hishida, H., 2007. Growth history and formation environments of ferromanganese deposits on the Phillippine Sea Plate, northwest Pacific Ocean. Isl. Arc 16, 420–430. http://dx.doi.org/10.1111/j.1440-1738.2007.00592.x.

Van Dover, C.L., **68**, 2010. Mining seafloor massive sulphides and biodiversity: what is at risk? ICES J. Mar. Sci., http://dx.doi.org/10/1093/icesjms/fsq086

Van Dover, C.L., Smith, C.R., Ardron, J., Dunn, D., Gjerde, K., Levin, L., Smith, S., 2012. Designating networks of chemosynthetic ecosystem reserves in the deep sea. Mar. Policy 36, 378–381. http://dx.doi.org/10.1016/j.marpol.2011.07.002.

Van Dover, C.L., Aronson, J., Pendleton, L., Smith, S., Arnaud-Haond, S., Moreno-Mateos, D., Barbier, E., Billett, D., Bowers, K., Danovaro, R., Edwards, A., Kellert, S., Morato, T., Pollard, E., Rogers, a, Warner, R., 2014a. Ecological restoration in the deep sea: Desiderata. Mar. Policy 44, 98–106. http://dx.doi.org/10.1016/j.marpol.2013.07.006.

Van Dover, C.L., 2014b. Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: a review. Mar. Environ. Res. 102, 59–72. http://dx.doi.org/10.1016/j.marenvres.2014.03.008.

Vanreusel, A., Hilario, A., Ribeiro, P.A., Menot, L., Arbizu, P.W., 2016. Threatened by mining, polymetallic nodules are required to preserve abyssal epifauna. Sci. Rep. 6, 26808.

Vardaro, M.F., Ruhl, H.A., Smith Jr., K.L., 2009. Climate variation, carbon flux, and bioturbation in the abyssal North Pacific. Limnol. Oceanogr. 54, 2081–2088. http://dx.doi.org/10.4319/lo.2009.54.6.2081.

Veillette, J., Sarrazin, J., Gooday, A.J., Galéron, J., Caprais, J., Vangriesheim, A., Étoubleau, J., Christian, J.R., Juniper, S.K., 2007. Ferromanganese nodule fauna in the Tropical North Pacific Ocean: species richness, faunal cover and spatial distribution. Deep. Res. Part I Oceanogr. Res. Pap. 54, 1912–1935. http://dx.doi.org/10.1016/j.dsr.2007.06.011.

Watson, R.A., Morato, T., 2013. Fishing down the deep: accounting for within-species changes in depth of fishing. Fish. Res. 140, 63–65.

Wattage, P., Glenn, H., Mardle, S., Van Rensburg, T., Grehan, A., Foley, N., 2011. Economic value of conserving deep-sea corals in Irish waters: a choice experiment study on marine protected areas. Fish. Res. 107, 59–67. http://dx.doi.org/10.1016/j.fishres.2010.10.007.

Wedding, L.M., Friedlander, A.M., Kittinger, J.N., Watling, L., Gaines, S.D., Bennett, M., Hardy, S.M., Smith, C.R., **280**, 2013. From principles to practice: a spatial approach to systematic conservation planning in the deep sea. Proc. Biol. Sci. . http://dx.doi.org/10.1098/rspb.2013.1684

Wedding, L.M., Reiter, S.M., Smith, C.R., Gjerde, K.M., Kittinger, J.N., Friedlander, a M., Gaines, S.D., Clark, M.R., Thurnherr, a M., Hardy, S.M., Crowder, L.B., 2015. Managing mining of the deep seabed. Science 349, 144–145. http://dx.doi.org/10.1126/science.aac6647.

Werner, S.R., Spurgeon, J.P.G., Isaksen, G.H., Smith, J.P., Springer, N.K., Gettleson, D. a, N'Guessan, L., Dupont, J.M., 2014. Rapid prioritization of marine ecosystem services and ecosystem indicators. Mar. Policy 50, 178–189. http://dx.doi.org/10.1016/j.marpol.2014.03.020.

White, C., Halpern, B.S., Kappel, C.V., 2011. Ecosystem service tradeoff analysis reveals the value of marine spatial planning for multiple ocean uses. Proc. Natl. Acad. Sci. USA 109, 4696–4701. http://dx.doi.org/10.1073/pnas.11114215109.

White, H.K., Hsing, P., Cho, W., Shank, T.M., Cordes, E.E., Quattrini, A.M., Roberts, H. H., Shedd, W., Reddy, C.M., Fisher, C.R., 2012. Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. Proc. Natl. Acad. Sci. USA 109, 20303–20308. http://dx.doi.org/10.1073/pnas.1118029109.

Williams, A., Schlacher, T.A., Rowden, A.A., Althaus, F., Clark, M.R., Bowden, D.A., Stewart, R., Bax, N.J., Consalvey, M., Kloser, R.J., 2010. Seamount megabenthic assemblages fail to recover from trawling impacts. Mar. Ecol. 31, 183–199. http://dx.doi.org/10.1111/j.1439-0485.2010.00385.x.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B. C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., 2006. Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787–791.

Xiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. 8, 593–599.

Yao, H., Dao, M., Imholt, T., Huang, J., Wheeler, K., Bonilla, A., Suresh, S., Ortiz, C., 2010. Protection mechanisms of the iron-plated armor of a deep-sea

hydrothermal vent gastropod. Proc. Natl. Acad. Sci. USA 107, 987–992. http://dx.doi.org/10.1073/pnas.0912988107.
Yasuhara, M., Doi, H., Wei, C., Danovaro, R., Myhre, S.E., **371**, 2016. Biodiversity-ecosystem functioning relationships in long-term time series and pelaeoecological records: deep sea as a test bed. Philos. Trans. R. Soc. B . http://dx.doi.org/10.1098/rstb.2015.0282.
Yesson, C., Clark, M.R., Taylor, M.L., Rogers, A.D., 2011. The global distribution of seamounts based on 30 arc seconds bathymetry data. Deep Sea Res. I 58, 442–453. http://dx.doi.org/10.1016/j.dsr.2011.02.004.
Ying, C.C., 1967. Learning by doing – an adaptive approach to multiperiod decisions. Oper. Res. 15, 797–812.
Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. Ecol. Econ. 64, 253–260. http://dx.doi.org/10.1016/j.ecolecon.2007.02.024.
Zhang, W., Swinton, S.M., 2012. Optimal control of soybean aphid in the presence of natural enemies and the implied value of their ecosystem services. J. Environ. Manag. 96, 7–16.

Acknowledgments

Chapter 2, in full, is a reprint of the material as it appears in Le, J.T., Levin, L.A., and R.T. Carson. 2017. Incorporating ecosystem services into environmental management of deep-seabed mining. Deep-Sea Research II. 137, 486-503. DOI: 10.1016/j.dsr2.2016.08.007. The dissertation author was the primary investigator and author of this material. The material is used by permission of Elsevier.

Chapter 3: Scientific and economic tradeoffs between morphological and molecular methods for biodiversity assessment in the deep sea

Jennifer T. Le, Lisa A. Levin, Franck Lejzerowicz, Tristan Cordier, Andrew Gooday, Jan Pawlowksi

Abstract

Deep-sea biodiversity is the source of various ecological functions and ecosystem services which are increasingly subject to human disturbances. However, current scientific tools may not be adequate for assessing and monitoring biodiversity in the face of emerging deep-sea industries such as mining. Here, we evaluate the scientific and economic tradeoffs associated with morphology-based taxonomy and metabarcoding in the context of nascent deep-seabed mining with a case study for polymetallic nodules in the Clarion-Clipperton Fracture Zone. We discuss types of information produced by these different methods, and use cost-effectiveness analysis to compare their relative abilities to yield useful results. While morphology-based taxonomy may be less cost-effective than metabarcoding (primarily due to associated labor costs), there are some scientific advantages associated with the former, such as generation of demographic data (densities, biomass, and size structure). Implementing an approach that combines the two methods at the beginning of commercial mining may facilitate future assessment and monitoring of impacts. Although our discussion here is specific to deep-seabed mining, these results are likely applicable to other habitats that are also data-limited and remote with few taxonomic experts.

Introduction

Deep-seabed mining is an emerging industry that could begin commercial production in the near future. It has potential to alter habitats targeted for minerals (abyssal plains, hydrothermal vents, seamounts) through physical disturbance, removal of substrate, sediment resuspension and deposition, light, and noise (Van Dover et al., 2014; Weaver et al., 2018; Christiansen et al., 2019). The International Seabed Authority (ISA), the body governing the international seabed and its resources (i.e. the "Area"), is tasked with developing rules, regulations, and policies, including those that will "[protect] the marine environment from harmful effects" and "[prevent] damage to the flora and fauna of the marine environment" (Article 145 of UNCLOS, 1982). This obligation highlights the need for two tasks: (1) establishing a baseline level of biodiversity for potentially impacted areas, and (2) monitoring changes in biodiversity due to mining activities against that baseline. Deep-sea biodiversity supports a range of ecological functions and ecosystem services, such as fish catch, genetic resources for industrial and pharmaceutical products, carbon sequestration and storage, and nutrient cycling (Armstrong et al., 2012; Thurber et al., 2014; Le et al., 2017).

The Clarion-Clipperton Fracture Zone (CCZ) is an abyssal plain area, ranging from approximately 3900-5500 m in depth, that hosts high densities of polymetallic nodules targeted for mining of copper, nickel, and cobalt. The CCZ has 30 exploration claims, each up to 75,000 square kilometers. It also contains a wealth of biodiversity, some associated with the nodules themselves (Veillette et al., 2007a; Gooday et al., 2015; Vanreusel et al., 2016). Pilot studies and mining simulations have found little recovery of Pacific abyssal plain habitats post-disturbance within 26 years (Miljutin et al., 2011; Jones et al., 2017; Gollner et al., 2017; Simon-Lledó et al., 2019). Common megafaunal taxa in the CCZ include ophuiroids, xenophyphores, and corals,

which are found in higher densities in the CCZ relative to other abyssal plain sites (Amon et al., 2016). Sediment macro- and meiofauna dominate eukaryotic species richness and densities on the abyssal plains (Rosli et al., 2018), and include foraminifera, nematodes, polychaetes, isopods, and tanaids (Wilson et al., 2017; Pape et al., 2017; Yu et al., 2018, Gooday & Goineau, 2019).

While current ISA draft regulation for commercial exploitation of the Area acknowledges biodiversity as something to measure, the measurement approaches are not specified. Biodiversity can be measured in a variety of ways: number of species in an area, species absolute and relative densities, number of functional roles in a system and species interactions, genetic variation between and among populations, representation of phylogenetic lineages, and number of habitats and ecosystems (CBD, 1993). Current faunal biomonitoring programs generally employ morphology-based taxonomy (MBT) (Baird & Hajibabaei, 2012), which requires an expert to manually sort and identify hundreds to thousands of individual organisms. As a result, taxonomic studies in the CCZ require a lot of resources (e.g. money, expertise, time).

The spatial heterogeneity of the CCZ biological community necessitates robust biodiversity assessment and monitoring (Wilson et al., 2017; Simon-Lledó et al., 2019). Large taxonomic gaps in deep-sea benthic communities still exist (Sinniger et al., 2016) despite scientists working tirelessly to identify known species and describe new ones. As a result, MBT is limited to animal taxa that not only exhibit distinguishing morphological features (excluding microbes and cryptic species), but also those for which expert taxonomists exist. Furthermore, this expertise can be especially difficult to find for deep-sea taxa and, as a result, much of the deep-sea environment remains undescribed (Ramirez-Llodra et al., 2010). MBT is strongly limited by the amount of time and cost required to generate data, and the lack of taxonomic

expertise that limits the breadth of biodiversity it can cover. This severely hampers scaling up both temporal and spatial resolution, and prevents timely adaptive management measures.

Although MBT is necessary in order to describe new species (which requires the deposition of holotypes into a collection), emerging molecular tools can serve to rapidly document biodiversity. Molecular techniques, such as metabarcoding and metagenomics, provide a rapid alternative for biodiversity measurements in natural systems (Taberlet et al., 2012; Stein et al., 2014; Porter & Hajibabaei, 2018). Technical advances have increased our capacity to generate biodiversity data, by moving from DNA extracted from a single individual to environmental DNA (eDNA) obtained from an environmental sample (e.g. sediment, water, soil, air) in order to rapidly assess whole biological communities. Here, we focus on the application of metabarcoding: the sequencing of specific (regions of) genes, used for taxonomic identification, in an environmental sample (Hebert et al., 2003).

A mix of both morphology-based and molecular-based methodologies are advocated to build robust and extended biodiversity inventories in the CCZ (Glover et al., 2016). An example of a combined workflow couples morphological identification of individuals with DNA sequencing (barcoding). While initially labor-intensive, this combined approach to taxonomy can facilitate later environmental assessment and monitoring by reducing the need for morphological identification as more species are described and sequenced. Additionally, a combined approach can promote standardization for data comparison among CCZ claims. For example, contractors measuring biodiversity within their own claims may be identifying the same species differently from other contractors. With a physical specimen associated with a genetic sequence, this issue could be more readily resolved even without a formal species description. Another example of a combined approach is initial genetic screening of large swaths of the CCZ (with metabarcoding)

to prioritize areas of interest for more detailed morphology work. It is likely that a combination of techniques is necessary in order to obtain scientifically robust data for environmental baseline and monitoring requirements set by UNCLOS and the ISA, such as those related to abundance and biomass, and genetic connectivity (ISA, 2019).

This paper aims to evaluate scientific and economic tradeoffs between MBT and metabarcoding of small eukaryotes (i.e. foraminifera, and metazoan meiofauna and macrofauna) in the context of deep-sea biodiversity assessment and monitoring. Specifically, we discuss MBT and metabarcoding for evaluation of deep-seabed mining impacts in the CCZ, where interest in polymetallic nodules is high (Hein & Petersen, 2013), small and rare taxa are dominant (Smith et al., 2006; Janssen et al., 2015; Wilson, 2017; Gooday & Goineau, 2019), and patchiness is substantial (Lins et al., 2016). We consider scientific tradeoffs between approaches in relation to environmental assessment and monitoring objectives, as well as how a combined approach can mitigate each method's weaknesses. Decision networks are constructed for each methodology to highlight how decisions within each approach can affect scientific outcomes and economic costs. Lastly, we assess and compare direct and indirect costs associated with each methodology in a cost-effectiveness framework.

Methods

Decision networks of morphology-based taxonomy and metabarcoding

Both MBT and metabarcoding techniques are framed here as a series of choices within a workflow that can influence both scientific outcomes and economic costs. We surveyed deep-sea experts and published protocols in order to determine the steps within each methodology, and how the choices within those steps affect scientific outcomes. In most cases, scientific questions

and desired outcomes (e.g. targeted taxa) dictate how choices are made, creating a range of appropriate protocols so only general steps are listed in the results.

Economic costs of biodiversity assessments

Cost-effectiveness analysis (CEA) is an economic approach that evaluates outcomes and their associated costs (Garber & Phelps, 1997). An action or policy can be considered "cost-effective" if (1) it is the least costly to obtain a desired outcome, or (2) it generates the best outcome given fixed resources. CEA differs from cost-benefit analysis because, rather than answering whether an action should be taken or not, it ranks strategies to maximize their efficiency (i.e. minimizes cost per unit of outcome). Posed with the question of how the ISA and contractors can meet environmental requirements, CEA can facilitate choosing which methods, or combination of methods, are most least-cost cost-effective.

For each methodology, experts and published protocols provided estimates of the consumables used and number of workhours taken in order to generate taxonomic data. Total cost (i.e. input) is the sum of consumable and labor costs. Fixed costs, such as laboratory equipment and bioinformatics pipelines, were not included in our analysis. Quantity of consumables (e.g. plastic tubes, chemicals) were summed. Prices (nominal 2016 USD) were taken directly from supplier websites, and are likely an overestimate because many research institutions receive discounted prices. Common suppliers in the U.S. were used: Fisher Scientific, VWR, and Qiagen, and prices were averaged among them. Labor costs came from best estimates of workhours (time spent actively transforming sediment samples into scientific data).

In our model, output is the number of "operational species" identified. "Operational species," or proxies for species, are commonly used in biodiversity assessments because sampled deep-sea organisms are often new to science (Rosli et al., 2018). MBT can employ morphospecies, individuals grouped together solely by morphology, whereas molecular methods use operational taxonomic units (OTUs) to distinguish species (Blaxter et al., 2005). Using operational species is less costly than describing every new species discovered, which involves writing a detailed morphological description and designating a holotype. Many taxonomic journals now insist on genetic data in addition. Combining morphological and genetic operational species (i.e. voucher specimen with associated sequence; Glover et al., 2016) may circumvent the need for formal species descriptions and provide a standard unit of outcome that is relevant to both decision-making and our analysis.

To approximate sampling regimes in the CCZ, we looked at a subset of published studies that attempt to characterize CCZ biodiversity of sediment eukaryotes using either MBT or molecular methods (Appendix 3A). Details from their sampling designs were extracted (e.g. vertical fraction depths, total number of samples used for analysis), as well as their relevant results (i.e. number of operational species identified). This information was used to make more appropriate comparisons between MBT and metabarcoding.

Results and discussion

Scientific tradeoffs between morphology-based taxonomy and metabarcoding

Scientific tradeoffs should be considered while comparing MBT and metabarcoding, including what data are generated and information gained from their interpretation. These are summarized in Table 3.1 and discussed below. CCZ biodiversity is dominated by small and rare

eukaryotes (Janssen et al., 2015; Wilson, 2017; Gooday & Goineau, 2019), which may favor taking a molecular approach to biodiversity assessments. However, although there is limited deep-sea taxonomic expertise, there is also little robust genetic information (e.g. sequences linked to species, genomes, transcriptomes) on CCZ fauna. As more sampling is completed, e.g. baseline surveys and during pilot mining programs, there will be an opportunity to build a database for genetic information that can be linked to specific species. Accomplishing this aim may require a combined approach.

Organism size-class

Benthic organisms can be classified by size: megafauna (visible to unaided eye), macrofauna (retained on a 300-µm mesh), meiofauna (retained on a 32-µm mesh), and microbial communities. Megafauna are the only group that can be readily identified with imagery generated by photo or video methods, which can also contribute to our understanding of organism behavior and interaction (Aguzzi et al., 2012). On the other end of the spectrum, microbial biodiversity is identified via molecular techniques such as sequencing (e.g. Sunagawa et al., 2015). Macro- and meiofauna fall somewhere in the middle, amenable to both approaches but perhaps both are not always simultaneously appropriate. Macrofauna are often used as environmental indicators (Dauvin, 2018), and there is work to develop similar deep-sea measures using meiofauna where they dominate the biological community (Laroche et al., 2015). It has been shown that MBT and metabarcoding can perform equally as well for biomonitoring of freshwater systems (Elbrecht et al., 2017) and assessment of marine aquaculture impacts (Lejzerowicz et al., 2015). In the deep sea, the limited taxonomic expertise on deep-sea

meiofauna (e.g. nematodes, soft-bodied foraminifera), may make the latter approach more appropriate for rapid assessment of biodiversity and environmental impacts.

Rare and cryptic species

Molecular methods are better suited for identifying rare and cryptic species than MBT. Because of their low abundance and patchy distribution, missing rare species because of under-sampling is often a problem, especially in the abyssal deep sea. Additionally, small, soft-bodied protists (e.g. flagellates and ciliates) often disappear in fixed sediment and only show up in eDNA (e.g. Sinniger et al., 2016). Although rare species do not have significant biomass, they can contribute unique functions (Chapman et al., 2018; Turner et al., 2019), and new species are discovered frequently with few representative individuals (e.g. Rouse et al., 2016; Golovan et al., 2017). Choosing clustering cutoffs for OTUs can affect results, losing rare species if too conservative and retaining technical artefacts (e.g. PCR error variant) if too lenient (Clare et al., 2017). Cryptic species usually cannot be identified via morphology and, therefore, require molecular methods to distinguish them. They have been documented in deep-sea taxa, such as polychaetes (Brasier et al., 2016) and gastropods (Johnson et al., 2015), and can significantly impact estimates of species richness (Brasier et al., 2016).

Demographic information, including abundance

MBT can provide demographic information (e.g. absolute density, morphological features, body size, biomass) and material that allow for examination of life histories, lifestyles, and functional attributes. On a practical level, sequencing technology cannot yet reveal these kinds of data. Whether demographic information is relevant to mining environmental

requirements depends on the goals and objectives set by the ISA (Tunnicliffe et al., 2019). Metrics such as density, dispersal, size structure, sex ratio, and physiological responses to environmental changes are often used to assess ecosystem health (Sidding et al., 2016). Metabarcoding is sufficient to estimate relative abundance, dispersal, and community changes (Rivera-Pinto et al. 2018), but additional techniques are required for the remaining metrics (i.e. MBT, other meta-omics). However, capacity to interpret molecular data continues to increase, such as experimental calibration to calculate abundance data (Darby et al., 2013), or computational techniques to identify differentially abundant taxa and community shifts (Morton et al., 2019).

Species

In order to establish identification of deep-sea species and their ecological roles, it is likely MBT and molecular approaches are needed in combination. Metabarcoding yields sequences of genes that are clustered into OTUs which can serve as a proxy for species. However, in order to assign an OTU to a species, that species must have been morphologically identified and sequenced previously, or belong to a taxonomic clade well-supported in a robust phylogeny (Washburn et al., 2018). Once described morphologically and genetically, metabarcoding alone can then be used to identify it. However, a molecular approach may greatly benefit from the improvement of MBT-based analyses, such as collating phylogenetic histories with environmental data (Washburne et al., 2019). The former is sufficient if species diversity is the only interest. However, ISA draft exploitation regulation (e.g. Regulation 2eiii) calls for the application of "an ecosystem approach" which implies information on functional diversity and ecological interactions are also necessary (ISA, 2019).

Time-sensitivity and expertise required

There could be scenarios in which rapid results are required in order to respond appropriately to environmental impacts, such as when an environmental threshold is surpassed or a trigger is set off indicating serious harm to the marine environment (Levin et al., 2016). An ecological threshold is a "tipping point" at which the system undergoes rapid and irreversible change that triggers an action (Groffman et al., 2006). As an example, a possible threshold to cessation of mining may be the loss of a specified amount of local biodiversity (Niner et al., 2018), implied by current mining guidelines for environmental management and monitoring plans (e.g. Part IX (Section 1), ISA, 2017; Annex VII (2j), ISA, 2019). In a scenario using MBT, results can be obtained only after months (or longer) of work, whereas metabarcoding results can be obtained within days after return to shore. This difference in response time may have significant impacts on the amount of environmental damage that ensues and, ultimately, the ability for an ecosystem to recover.

The amount of time required to develop the expertise necessary for either approach differs greatly. Depending on taxon, it can take multiple years for a taxonomist to become accurate and efficient at species identification using MBT. In the deep sea, this expertise is especially lacking, particularly for small taxa, which can overwhelm experts' workloads and increase turnaround time. Parataxonomy, which is the delegation of taks in an MBT workflow to non-experts, can alleviate some pressure on taxonomic experts. Although parataxonomy does not yield reproducible results, it can be useful when rapid biodiversity assessments are necessary (Abadie et al., 2008). In contrast, popularity of molecular approaches has caused the proliferation of sequencing facilities, both academic and commercial, for turnaround time of approximately 2-

3 weeks from sample submission to data return. The more limited training required to process sequencing samples makes molecular approaches more accessible, and the automation that is actively being developed will further reduce processing times. Additionally, loss of taxonomic expertise over time can be mediated by genetic information which can be used as an immutable characteristic for taxonomic identification.

Some deep-sea methodology challenges

One of the biggest challenges facing both MBT and molecular approaches is developing an adequate sampling design. There are still many gaps in our knowledge of deep-sea biodiversity (Sinniger et al., 2016; Shulse et al., 2017), but it is unclear at what spatial and temporal scales scientists need to sample in order to accurately characterize it (Lejzerowicz et al., 2015; Leduc et al., 2015). Spatial heterogeneity is poorly defined and likely differs among habitat and location. In the CCZ, there can be unexpectedly high spatial heterogeneity (Veillette et al., 2007b; Simon-Lledó et al., 2019). Additionally, adequate temporal resolution of sampling for accurate characterization of deep-sea habitats is unknown (Yasuhara et al., 2009). MBT and metabarcoding often provide one snapshot in time and, given time constraints associated with MBT, a comprehensive monitoring program may incorporate both methodologies by employing metabarcoding more frequently and MBT at longer intervals.

The study of deep-sea habitats is plagued by a lack of data, creating challenges that are exacerbated by the sheer vastness of the deep sea. The CCZ claim areas span almost 4500 km wide, larger than the continental U.S., which leads to high levels of uncertainty because of data limitation. A precautionary approach, wherein adequate measures are taken in order to manage risk, should be invoked (Rio Declaration, 1992; ISA, 2018). Therefore, collection of robust

baseline data is critical for protection of the marine environment (Durden et al., 2018), as well as exhaustive monitoring, especially at the early stages of commercial exploitation (e.g. pilot mining). MBT and molecular approaches can be utilized together in these programs to facilitate future environmental assessment and monitoring.

Economic tradeoffs between morphology-based taxonomy and metabarcoding

There are direct and indirect costs involved in collecting and processing sediment samples for MBT and metabarcoding. Here, they are separated into costs associated with consumables (direct) and labor (indirect). Other costs, such as laboratory equipment (capital) and bioinformatics pipelines, are held fixed. Choices within the decision networks are discussed in terms of how they affect the short-run economic cost. We assume that laboratories have basic amenities and access to people who are trained to do these tasks, i.e. identifying target taxa, preparing sediment samples for sequencing.

Sampling costs

Deep-sea samples, whether sediment, water, or individual organisms, are relatively expensive to collect because they often require research expeditions on global-class vessels equipped with specialized instruments. One ship-day on a U.S. vessel can range from $42,000-48,000 USD, which includes food, fuel, crew, and two technicians (personal communication, E. Brenner). The amount of time it would take to sail from the Scripps Institution of Oceanography pier (San Diego, USA) to the eastern edge of the CCZ is approximately six days at full speed without stopping, resulting in over a quarter million dollars in transit time one-way. This cost estimate does not include the use of autonomous underwater vehicles (AUVs), remotely-operated

vehicles (ROVs), or human-occupied vehicles (HOVs), commonly used on deep-sea expeditions. In addition to collecting sediment samples for both MBT and sequencing, deep submergence vehicles can conduct visual surveys. The U.S. National Deep Submergence Facility (NDSF) operates their instruments at day-rates of $14,000 for AUV *Sentry*, $23,000 for ROV *Jason*, and $45,000 for HOV *Alvin* (NDSF, 2018). Other sampling equipment includes box corers, multi-corers, epibenthic sleds, and CTD rosettes which can be deployed without special vehicles.

Relevant results and sampling regimes from studies included in this paper are summarized in Table 3.2. Sediment samples were taken with multi-corers, box corers, or an epibenthic sled in one case, and the same sampling devices can be used for both approaches. The total number of samples used for analysis ranged from 5-41 and 15-42 per cruise for MBT and metabarcoding, respectively. Here, we assume number of samples equates to number of vertical fractions (core slices). Sampling costs are likely the biggest element but should not contribute significantly to cost differences between the two analytical approaches. However, the different methods used to process deep-sea sediment samples are a major factor.

Processing costs for morphology-based taxonomy

Figure 3.1 outlines the general steps and choices within an MBT workflow. Most decisions are dependent on target taxa, which dictates sieve sizes and the number of vertical fractions. Sediment cores are taken via remotely-operated vehicle or multi-corer. Cores are then sliced into vertical fractions (e.g. 0-1cm, 1-2cm, etc.), and commonly preserved in dilute formalin or ethanol. Samples in ethanol can subsequently be used for molecular analysis. Dependent on target taxa, appropriate sieves are used to separate organisms from sediments. Meiofauna are generally sieved on 32 (nematodes), 42, 63 (foraminifera), or 150 (foraminifera)

μm mesh whereas macrofauna are retained on 0.25 or 0.3 mm sieves. Finer sieves take longer to process than coarser ones (Gooday & Goineau, 2019). Sieved samples can then be sorted to identify and quantify animals. As the number of samples, vertical fractions, and level of identification increases, the data resolution also increases, but at the expense of time. For comparison, the commercial cost of sorting and identifying shallow-water macrofauna as one metric of aquaculture impacts from a standard Van Veen grab sample varies from 500 to 1500 USD (personal communication, J. Pawlowski).

Consumables required for MBT are common to most labs and are readily available (Appendix 3B). Preservation method can have significant effects on downstream options. Formalin is cheaper, easier to ship (non-hazardous at 4%), and fixation results in little distortion of morphology. However, specimens fixed in ethanol can later be used for molecular studies which is advantageous and cannot be done with specimens fixed in formalin. The total cost of lab consumables for MBT ranges from $13.10-16.29 USD.

Labor and expert time are the dominant costs of the MBT workflow (Appendix 3C). Based on a graduate student stipend in the U.S. of $29,500 USD annually, time alone ranges from $325-2,875 per sediment core slice. Additionally, the estimates shown here are only for the taxa listed for one vertical fraction of a sediment core although the top several centimeters are likely necessary for biodiversity characterization. The extensive time needed to manually sort and identify organisms, especially when using smaller sieve sizes that retain more sediment, is a large cost and a disadvantage of using MBT. Ludox® flotation, a method of concentrating animals in a liquid medium, can reduce picking and sorting times but adds additional consumable costs. The estimates we have provided are based on hand-sorting.

Processing costs for metabarcoding

There are many options when processing marine sediment for metabarcoding (Figure 3.2), influencing scientific outcomes and economic costs. A metabarcoding approach can also be used in parallel with other omics methods (e.g. metagenomics, metatranscriptomics) if relevant to objectives (e.g. Franzosa et al., 2015).

The number of replicates in a study can refer to either sample replicates or PCR replicates. If there is enough sediment material in each sample, then pseudo-replicates (multiple samples from the same core) can be added. PCR replicates are more often used and can help minimize errors due to PCR, such as unequal amplification and chimeras (Acinas et al., 2005). Additional replicates yield more robust results and higher statistical power, but increase total cost (i.e. because more sediment is being processed and sequenced). Sampling replicates and pseudo-replicates can also apply to MBT.

Metabarcoding utilizes highly conserved fragments of DNA that all organisms have and within these fragments are markers, which are highly variable regions that allow for higher resolution taxonomic classification. Choosing markers, how many and which ones, can affect results (Clarke et al., 2017) and will depend on scientific objectives. 16S rRNA can be used to assess microbial diversity (e.g. Salazar & Sunagawa, 2017) whereas eukaryote diversity is typically assessed with markers within the 18S rRNA or cytochrome oxidase I genes (e.g. Zaiko et al., 2015; Gimmler et al., 2016). More comprehensive diversity assessments may require multiple markers (e.g. Evans et al., 2016) in order to completely capture the community. Advantages to working with widely-used markers include a more optimized protocol, the ability to compare to other studies, and a larger database. However, there is no truly universal marker that allows the retrieval of all taxa without bias. Additionally, a universal marker must be very

conserved and therefore less useful for species-level taxonomic assignments. Development of markers targeting indicator taxa allows for better detection of them (Zhang et al., 2018). These bioindicators can then be used for characterizing specific (micro)habitats, such as sediment plumes, and early detection of mining impacts (Fukushima & Nishijima, 2017).

Molecular primers initiate DNA replication and are used in metabarcoding approaches to amplify targeted barcodes. However, primer specificity (i.e. how accurately primers attach to targeted binding regions) is taxon-specific and introduces bias during PCR. This can lead to the masking of some taxa and inaccurate estimates of relative abundance (Elbrecht & Leese, 2015; Leray & Knowlton, 2015; Tremblay et al., 2015). Use of multiple primers that target the same taxonomic group can help minimize primer bias and increase taxonomic coverage (Alberdi et al., 2018). Primers can target specific taxa, such as indicator species from which extensive diversity has been catalogued (Morard et al., 2018), single-copy genes such as cytochrome oxidase I (Leray et al., 2013), or both for higher accuracy (Kosakyan et al., 2015).

Tags or indexes (i.e. the series of nucleotides at the start of each primer that labels PCR products) can contribute significantly to total costs, e.g. if many different primers are necessary, if the primers are tagged only with a short sequence (Esling et al., 2015), or if these primers include all accessory sequences required for library indexing and sequencing (Kozich et al., 2013). Primers that include a tag sequence may bias sequence counts. This can be addressed with additional PCR replicates (O'Donnell et al., 2016). Number of libraries and library preparation can also comprise a large portion of the cost. Each library can cost approximately $50 USD on the higher end. Libraries prepared using long primers may also need to be PCR-amplified and quantified using qPCR which can be expensive. Hence, there are trade-offs in defining the most

efficient and economic sequencing strategy, which mainly consists of balancing the numbers of PCR tagging and library indexing experiments to perform.

Advances in sequencing technology have facilitated the wider application of molecular methods such as environmental assessment and monitoring (Porter & Hajibabaei, 2018). Illumina platforms (HiSeq, MiSeq) are currently the most widely used. Other platforms include those from Pacific Biosciences (single-molecule real time sequencing), Life Technologies (SOLiD, ion torrent), and Oxford Nanopore (nanopore). Research groups that routinely sequence samples may buy their own sequencing machine, whereas other groups may send samples to a company to sequence. Sequencing costs are charged per lane or per cell, depending on the platform used. Samples can be pooled to run in one lane so that more samples can be sequenced at a lower cost. However, this yields less reads per sample. Samples are often processed in batches, e.g. 14.7 workhours per one 96-well plate, and although sequencing itself takes hours, it need not require active workhours (Appendix 3E).

There are also scientific tradeoffs among the platforms and the most suitable is likely dependent on the project. For example, in comparing the Illumina HiSeq and Miseq machines, the MiSeq yields relatively longer read lengths whereas the HiSeq yields more reads. These features may make the MiSeq more fitting for targeted sequencing (i.e. metabarcoding) because longer reads can better capture barcodes. In contrast, the HiSeq may be more fitting for metagenomics because more material can be sequenced, yielding more reads. This may be critical for metabarcoding studies with numerous samples of highly diverse communities that necessitate more sequencing depth in order to describe the biological community.

The cost of molecular methods is driven largely by kits and chemicals that are necessary to preserve and extract samples, as well as sterile equipment to prevent contamination (Appendix

3D). Several protocols were referenced to determine the cost of consumables, ranging from $24-49 USD (Bourlat et al., 2016; Fonesca & Lallias, 2016; personal communication, T. Cordier). For labs that routinely prepare and sequence many samples, these consumable costs are likely reduced. The reaction volume for PCR and library preparation can be reduced and saved volume can be redistributed to other samples. Labs can also prepare their own chemical reagents to reduce costs. Sequencing costs are declining over time (Weymann et al., 2017), facilitating use of molecular methods. In 2012, Illumina sequencing ranged from $41-502 per Gb (Quail et al., 2012). Barcodes are generally 400-800 bp, comprising a small portion of total cost individually but there may be millions of reads produced (e.g. Illumina MiSeq has a maximum of 25 million reads per run).

Bioinformatics and bioinformaticians are needed to analyze and understand the large amounts of biological data that metabarcoding can produce. This stage often includes creating a pipeline, or series of scripts, that automates quality-control and analysis of data (e.g. Brandt et al., 2019). Pipelines can have downsteam impacts on data interpretation and should be catered to scientific objectives (Pauvert et al., 2019). Although labs can have dedicated bioinformatics personnel, there are also companies and organizations that provide these services (e.g. centers based at universities). Hourly and daily rates range: Oregon State University (USA) charges $80-160 USD per hour, the University of Texas (USA) charges $90-110 USD per hour, and Indiana State University (USA) charges $460-690 USD per day. In the context of biodiversity assessment and monitoring for deep-seabed mining impacts, the economic costs associated with bioinformatics will likely be a one-time cost to create the pipeline which can then be used on subsequent data collected. As a result, we consider these costs fixed and did not include them in

our analysis. Well-defined scientific objectives could help minimize these costs by providing details on expected outcomes.

Overhead charges, which are expenses associated with day-to-day costs such as building rent and utilities, can range from 37-120% of direct costs. The lower end is associated with universities and academic institutions, whereas the higher end may be charged by private companies.

Cost-effectiveness analysis

Here, short-run, variable total cost (TC) for either MBT or metabarcoding (denoted with "meta") is the sum of consumable and labor costs (Equations 3.1 and 3.2), whereas benefits are operational species identified (i.e. 100-416 morphospecies or 451-706 OTUs; Table 3.2).

(Equation 3.1)

$$TC_{MBT} = (lab\ consumable\ costs_{MBT} + labor\ costs_{MBT}) \times number\ of\ samples_{MBT}$$

(Equation 3.2)

$$TC_{meta} = (lab\ consumable\ costs_{meta} + labor\ costs_{meta}) \times number\ of\ samples_{meta} + (sequencing\ costs \div number\ of\ samples_{meta})$$

Table 3.3 summarizes values of each category of cost and chosen distribution models, which are used to incorporate variability among workflows and uncertainty (Halpern et al., 2006). There are few variables, but many options, in MBT consumables and so a uniform distribution is used. MBT labor is highly variable, depending on expertise, size-class of organism (smaller taxa generally take longer), and level of identification. A low and high estimate are

given, based on expert response, and the mean is used as the value with the highest probability in a triangle distribution. The opposite is the likely case for metabarcoding in which labor is constant but consumables can vary depending on the decisions discussed above. Additionally, there are sequencing costs which can vary with sequencing platform and DNA yield. Sequencing costs here are from Cornell University commercial services.

We performed Monte Carlo simulations (100,000 iterations). Median cost to identify an operational species (without a formal description) with MBT was $92.62 (SD $111.97) and $7.62 (SD $6.07) for metabarcoding. The marginal cost of identifying a new species is likely to increase as more species are identified because it becomes more difficult to collect rare species that have not yet been discovered. However, the marginal cost of identifying a previously-described species decreases over time as more species are discovered. Expected mean efficiency (i.e. output divided by input) for metabarcoding (0.185) was an order of magnitude greater than MBT (0.016).

These results are most sensitive to MBT labor, but also show trends with metabarcoding consumables and labor. There is a large range of MBT labor costs, dependent on target taxa and level of identification, which also drives the majority of total cost. Technology that could reduce MBT labor costs, such as artificial intelligence, could increase the cost-effectiveness of MBT. Range in metabarcoding consumable costs is related to chemical kits, some of which can be made in-house, that are necessary for preservation and extraction.

There is some overlap between cost and efficiency ranges of the two approaches as seen in Figure 3.3. However, there was only a 0.028 probability that metabarcoding was more expensive per operational species than MBT. For the same cost, metabarcoding yields 12 times more identified operational species. This is likely appealing for contractors that are required to

collect baseline data. Measurements, such as the Chao 1 estimator (Chao, 1984) and rarefaction curves, can help gauge whether species in an area have been adequately sampled.

Borrell et al. (2017) presented similar results as these in their comparison of approaches for detecting shallow water invasive species. They include visual examination, DNA barcoding, and metabarcoding of water samples in their evaluation of consumable and labor costs. The authors find that the labor necessary for visual examination makes it the most expensive option. In the case of mining in the CCZ, this disparity between labor costs is even greater because of the need to identify all morphospecies (i.e. for a comprehensive biodiversity assessment) rather than targeted invasive species. The development of indicator species for mining impacts may help lower labor costs for MBT and also molecular approaches. Indicators for essential deep-sea ecological processes and ecosystem services can also be developed for a more holistic, ecosystem-based approach (Le et al., 2017; ISA, 2018).

Use of only molecular methods may not be sufficient to establish adequate baselines currently. The Glover et al. (2016) combined approach links morphospecies with genetic barcodes to yield robust operational species that can be used across claim areas and over time. As more morphospecies are vouchered and associated with genetic barcodes, the need for taxonomic identification via morphology decreases because species can be identified with cost-effective sequencing. This approach requires a large investment of resources (e.g. funding, time, expertise) to build reference databases initially, but phases out the need for MBT over time to use solely molecular methods, which are continuing to decrease in cost. This combined approach has a total cost of both methods:

(Equation 3.3)

$$TC_{combined} = TC_{MBT} + TC_{meta}$$

Although Glover et al. (2016) use targeted barcoding, here we use our estimates for metabarcoding because the cost of picking organisms from sediment samples, necessary for targeted barcoding, is already incorporated into the MBT cost. There may be additional costs associated with reconciling morphological and genetic data that are not incorporated here. Equation 3.3 yields a median cost per operational species of $72.29 (SD $97.40), comparable to MBT cost alone. However, a combined approach provides the additional benefit of creating a comprehensive database of taxonomic information that can be used among claims and over time in order to ensure comparison of the same biological communities. As a result, future assessment and monitoring could comprehensively rely on molecular methods alone because genetic information will be associated with a voucher specimen or (morpho)species that can be referred to and updated, if necessary.

While Glover et al. (2016) represents a completely combined approach in which both morphological and targeted genetic analyses are applied to each sample, there could be scenarios in which the total number of samples are split between the two approaches. For example, if the number of operational species is the only goal, then it would be most cost-effective to allocate all resources to metabarcoding. However, we have presented some scientific advantages to employing MBT in previous sections. The tradeoffs between number of operational species identified and additional scientific information can be represented by the following:

(Equation 3.4)

$$TC_{combined} = \rho TC_{MBT} + (1 - \rho) TC_{meta}$$

Where ρ is a weight determined by stakeholders (e.g. the ISA, contractors, scientists) and how they value outcomes. This may change over time as more operational species are identified.

Similar to the other combined case, there are diminishing marginal returns on MBT: as more operational species are characterized by morphology, it becomes less difficult to identify species genetically.

Conclusions

Several scientific tools can address biodiversity assessment and monitoring of mining impacts, such as MBT and molecular methods (here, metabarcoding). Each of these approaches have specific scientific and economic tradeoffs associated with them. While MBT is relatively expensive due to labor costs, it provides information on demographic factors, such as absolute density, biomass, size structure, or life history, that metabarcoding cannot. However, genetic sequencing continues to decrease in cost and increase in data interpretation. Using operational species as a measure of outputs, metabarcoding can be more cost-effective than MBT, largely due to labor costs associated with the latter.

The International Seabed Authority must protect the marine environment from serious harm (Article 145, UNCLOS 1982) which requires robust environmental baselines and thorough monitoring of impacts. An investment to build our knowledge of deep-sea biodiversity, e.g. through a combined MBT and molecular approach, could facilitate future assessment and monitoring by allowing contractors to more easily identify species. It will also aid comparison among contractor claims, and through time by presenting immutable characters (i.e. DNA). How the ISA addresses biodiversity in environmental rules, regulations, and policies will dictate how, and if, these scientific tools are used (e.g. Tunnicliffe et al., 2019). Stakeholder buy-in will be necessary for their adoption. Baseline data are critical to understanding of how mining activity can impact deep-sea ecosystems. The ISA has a novel opportunity to establish robust data

collection protocols to develop a mining industry that explicitly considers environmental impacts on the wealth of biodiversity in deep-sea systems, essential to key functions and services that benefit humankind as a whole (Le et al., 2017).

Although this study was done in the context of deep-seabed mining in the CCZ, the results are likely similar across marine, aquatic, and terrestrial systems that are (1) data-limited and (2) not easily accessible, e.g. other deep-sea habitats, caves, and other remote areas. Difficulty and expense in obtaining samples make each one valuable in terms of the information it can provide, which may point to benefits of a more comprehensive analysis, at least when building taxonomic databases. Scaling up these approaches necessitates communication among stakeholders and standardization of data collection with potential gains in meeting environmental requirements with transparency and relatively low uncertainty.

Acknowledgements

This work was supported by the Swiss Network for International Studies award (20170024), the University of California San Diego Frontiers of Innovation Scholars Program (g001), and the Deep-Ocean Stewardship Initiative for funding to attend the International Seabed Authority Session. The authors would like to thank Ann Vanreusel, Carlos Neira, and Holly Bik for their significant input regarding methodologies; and Dale Squires, Richard Carson, Gregory Rouse, and Eric Allen for their invaluable advice in preparing this manuscript.

Chapter 3, in part, is in preparation for submission for publication. Le, J.T., Levin, L.A., Lejzerowicz, F., Cordier, T., Gooday, A., and J. Pawlowski. Scientific and economic tradeoffs between morphological and molecular methods for biodiversity assessment in the deep sea. The dissertation author was the primary investigator and author of this material.

Tables

Table 3.1. Advantages and disadvantages associated with information gained using morphology-based taxonomy and metabarcoding for use in deep-sea biodiversity assessment and monitoring.

Information category	Morphology-based taxonomy	Metabarcoding
Small-size classes	Possible but difficult	Better suited
Rare & cryptic species	Poorly suited	Better suited
Abundance (density)	Absolute	Relative
Morphological data	Can provide	Cannot provide
Demographic information (e.g. biomass, size, age, sex)	Can provide	Cannot provide
Behavior	Cannot provide	Cannot provide
Species	Identified by experts	Barcode matched to database
Time to results	Weeks to months	Days
Expertise required	Years of training	Weeks of training
Biases	Human error	Technical error

Table 3.2. Summary of relevant results and sampling regimes of published papers aimed at characterizing a component of meio- and macrofaunal biodiversity in the Clarion-Clipperton Fracture Zone. Papers are listed in Appendix 3A.

Sampling component	Morphology-based taxonomy	Metabarcoding
Number of sites visited	1-6	1-11
Deployments per site	1-26	1-12
Cores used per deployment	1-3	1-3
Sub-samples per core	1-3	1-3
Number of horizontal layers	1-5 fractions*	1-3 fractions
Total number of samples	5-41	15-42
Operational species identified	100-416	451-706

*Top 1-2 cm for foraminifera, top 5 cm for other meiofauna, top 10 cm for macrofauna

Table 3.3. Variables used in the cost-effectiveness model, their high and low estimates, and distributions chosen to incorporate risk and uncertainty. A uniform distribution assigns the same probability to every value within its given range. A triangular distribution assigns the highest probability to, here, the mean of the high and low estimates. MBT refers to morphology-based taxonomy.

Variable description	High estimate	Low estimate	Distribution
MBT consumables	16.29	13.10 (without staining)	Uniform
MBT labor (in hours)	202.8 per core slice (foraminifera)	22.8 per core slice (meiofauna)	Triangular
Molecular consumables	49.45	10.09 (in-house chemicals)	Triangular
Molecular sequencing	1800 per run	900 per run	Triangular
Molecular labor (in hours)	14.7	14.7	Constant

Figures

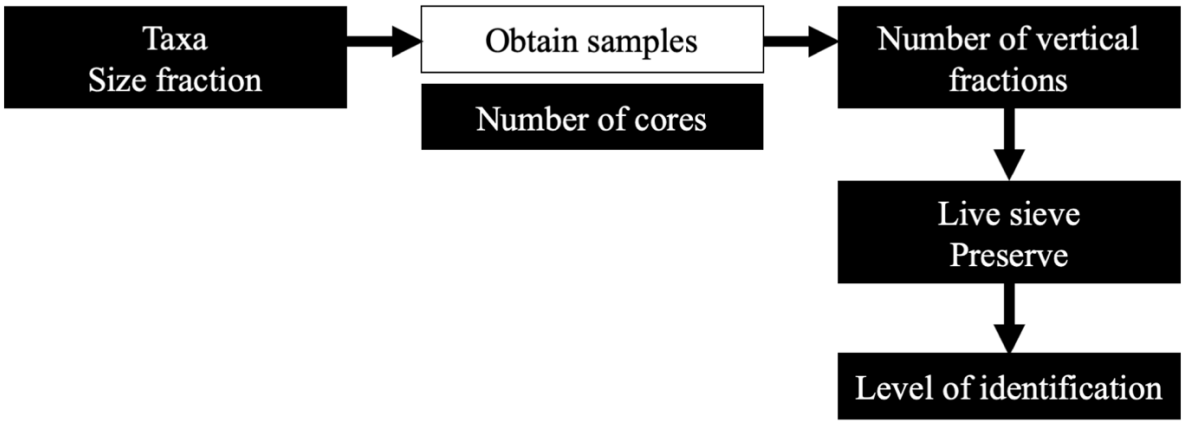

Figure 3.1. Decision network throughout a morphology-based taxonomy workflow. Decision nodes are black and steps that do not require a decision are unfilled.

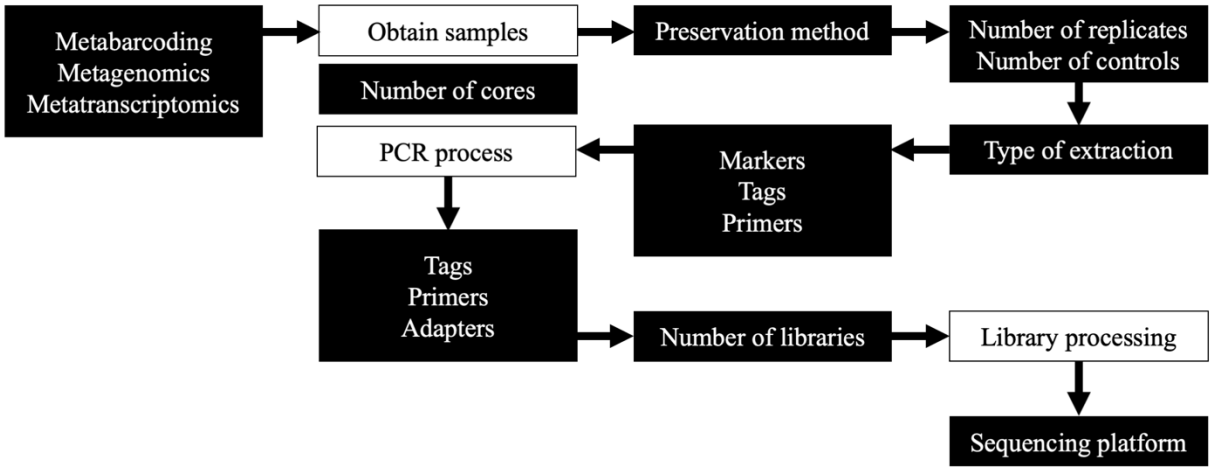

Figure 3.2. Decision network throughout a metabarcoding workflow. Decision nodes are black and steps that do not require a decision are unfilled.

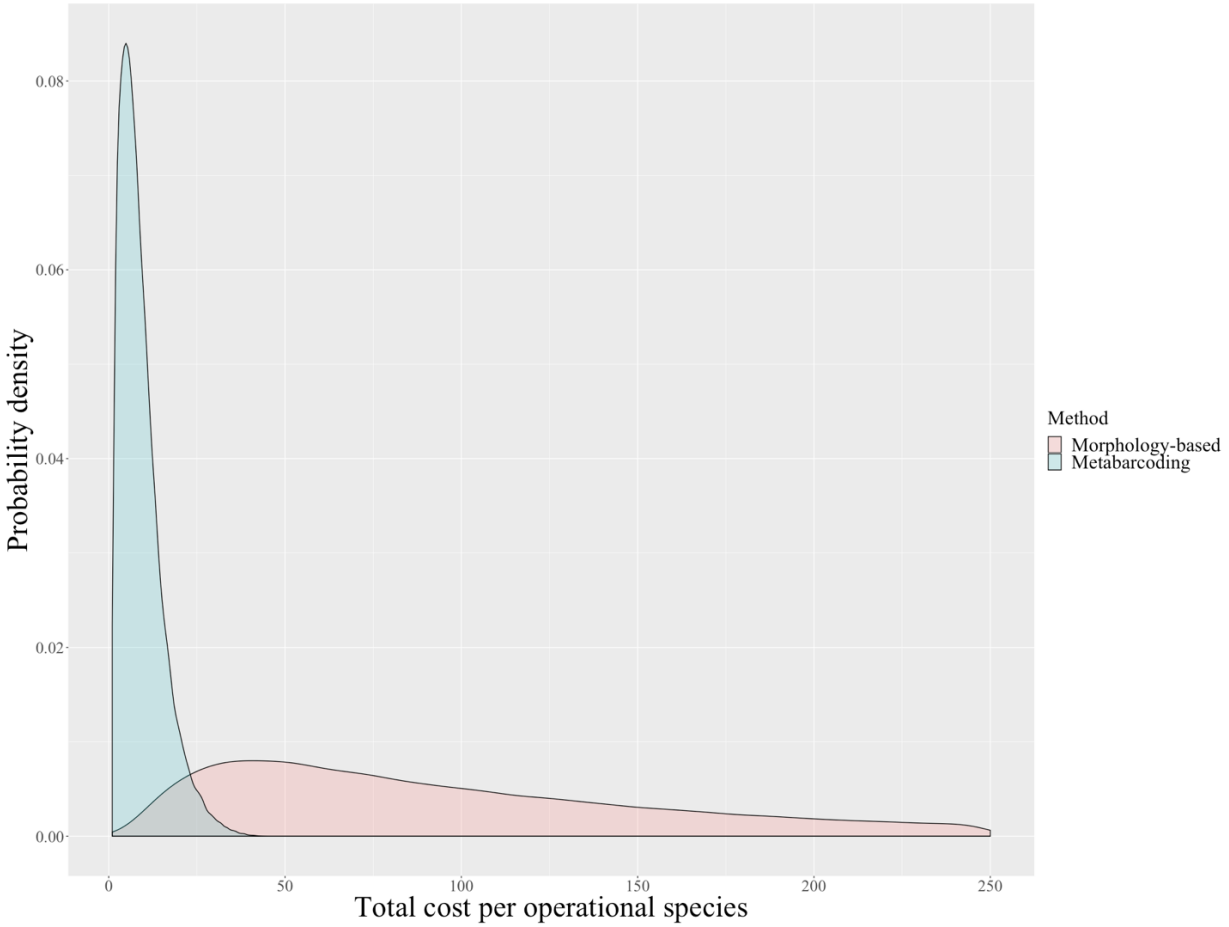

Figure 3.3. Probability density estimates of expected total cost per operational species of morphology-based taxonomy (pink, truncated) and metabarcoding (blue).

Appendices

Appendix 3A. List of studies on biodiversity in the Clarion-Clipperton Fracture Zone that were used to examine sampling schemes in order to make comparisons between morphology-based taxonomy and metabarcoding. Full citations are in the References section.
De Smet et al., 2017; Goineau & Gooday, 2017; Hauquier et al., 2018; Janssen et al., 2015; Lambshead et al., 2003; Lejzerowicz et al., 2014; Miljutin et al., 2011; Miljutina et al., 2010; Pape et al., 2017; Wang et al., 2010; Wilson et al., 2017; Yu et al., 2018

Appendix 3B. The average cost of consumables to identify individuals of one morphospecies in a vertical fraction of a sediment core. Prices were obtained directly from supplier websites and are likely higher than what an academic institution would pay due to institutional discounts. n = the number of suppliers used to calculate the average cost.

Item	**Average cost per unit** (USD)	**Average cost per sample** (USD)	**n**
Stain (Rose Bengal)*	2.91 per g	2.91	3
Formalin 37%	0.12 per ml	6.24	3
Plastic jars	5.68 per jar	5.68	3
Glass vials	0.63 per vial	0.63	3
Pasture pipettes	0.15 per pipette	0.15	3
Transfer pipettes	0.13 per pipette	0.13	3
Cryovials	0.55 per vial	0.55	3
Total average cost of consumables = 13.10 – 16.29			

*Staining commonly used for foraminifera and other meiofauna.

Appendix 3C. Estimate of active time for each step in a morphology-based taxonomy workflow.

Task	Workhours
Core recovery	0.25
Core slicing	0.75
Core preservation	0.3
Core splitting	1
Core sieving	0.5
Sample sort & identify meiofauna	20-200
Total time for foraminifera = 202.8 workhours Total time for meiofauna = 22.8 workhours	

Appendix 3D. Average cost of consumables to process one sediment sample for metabarcoding in nominal USD, rounded to the nearest cent. n = the number of suppliers used to calculate the average cost.

Item	**Average cost per unit** (USD)	**Average cost per sample** (USD)	n
LifeGuard Soil Preservation Solution	1.92 per ml	9.62	1
PowerSoil DNA Extraction Kit	5.14 per sample	5.14	1
PowerMax DNA Isolation Kit	24.60 per sample	24.6	1
QIAxcel DNA Screening Kit	0.43 per sample	0.52	1
QX size marker 50	0.03 per sample	0.03	1
QX alignment marker	0.04 per sample	0.04	1
Ethanol	0.06 per ml	0.06	3
5M NaCl	0.07 per ml	0.01	3
Tubes	0.27 per tube	1.55	3
Pipette tips	0.23 per tip	3.02	3
Sterile gloves	3.54 per pair	3.54	3
Sterile scoops	1.31 per scoop	1.31	3
Total average cost of consumables = 49.45			

Appendix 3E. Estimate of active time for each step in a metabarcoding workflow.

Task	Workhours
DNA extraction	4
DNA precipitation	0.5
DNA stock/aliquot	1
PCR amplification	1
QIAxcel quantification	0.5
Pool	0.5
Pool evaporation	0.25
Pool purification	0.5
Pool quantification Qubit	0.5
Library preparation	3
Library quantification by qPCR	2
Library verification by gel	0.45
Library dilution	0.5
Bioinformatics analysis	variable
Total time = 14.7 workhours (per sample but these are generally done in batches of up to 96)	

References

Abadie, J. C., C. Andrade, N. Machon, and E. Porcher. 2008. On the use of parataxonomy in biodiversity monitoring: A case study on wild flora. Biodiversity and Conservation 17:3485–3500.

Acinas, S. G., R. Sarma-rupavtarm, V. Klepac-ceraj, and M. F. Polz. 2005. PCR-Induced Sequence Artifacts and Bias : Insights from Comparison of Two 16S rRNA Clone Libraries Constructed from the Same Sample. Applied and Environmental Microbiology 71:8966–8969. DOI: 10.1128/AEM.71.12.8966.

Aguzzi, J., J. B. Company, C. Costa, M. Matabos, E. Azzurro, A. Mánuel, P. Menesatti, F. Sardá, M. Canals, E. Delory, D. Cline, P. Favali, S. K. Juniper, Y. Furushima, Y. Fujiwara, J. J. Chiesa, L. Marotta, N. Bahamon, and I. G. Priede. 2012. Challenges to the assessment of benthic populations and biodiversity as a result of rhythmic behaviour: Video solutions from cabled observatories. Oceanography and Marine Biology: An Annual Review 50:235–286.

Alberdi, A., O. Aizpurua, M. T. P. Gilbert, and K. Bohmann. 2018. Scrutinizing key steps for reliable metabarcoding of environmental samples. Methods in Ecology and Evolution 9:134–147. DOI: 10.1111/2041-210X.12849.

Amon, D. J., A. F. Ziegler, T. G. Dahlgren, A. G. Glover, A. Goineau, A. J. Gooday, H. Wiklund, and C. R. Smith. 2016. Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. Scientific Reports 6:30492. DOI: 10.1038/srep30492.

Armstrong, C. W., N. S. Foley, R. Tinch, and S. van den Hove. 2012. Services from the deep: Steps towards valuation of deep sea goods and services. Ecosystem Services 2:2–13.

Baird, D. J., and M. Hajibabaei. 2012. Biomonitoring 2.0: A new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. Molecular Ecology 21:2039–2044. DOI: 10.1111/j.1365-294X.2012.05519.x.

Blaxter, M., J. Mann, T. Chapman, F. Thomas, C. Whitton, R. Floyd, and E. Abebe. 2005. Defining operational taxonomic units using DNA barcode data. Philosophical Transactions of the Royal Society B: Biological Sciences 360:1935–1943.

Borrell, Y. J., L. Miralles, H. Do Huu, K. Mohammed-Geba, and E. Garcia-Vazquez. 2017. DNA in a bottle - Rapid metabarcoding survey for early alerts of invasive species in ports. PLoS ONE 12:e0183347. DOI: 10.1371/journal.pone.0183347.

Bourlat, S.J. 2016. Preparation of Amplicon Libraries for Metabarcoding of Marine Eukaryotes Using Illumina MiSeq: The Dual-PCR Method. In: Bourlat S. (eds) Marine Genomics. Methods in Molecular Biology 1452. Humana Press, New York, NY.

Brandt, M. I., B. Trouche, L. Quintric, P. Wincker, J. Poulain, and S. Arnaud-Haond. 2019. A flexible pipeline combining bioinformatic correction tools for prokaryotic and eukaryotic metabarcoding. bioRxiv.

Brasier, M. J., H. Wiklund, L. Neal, R. Jeffreys, K. Linse, H. Ruhl, and A. G. Glover. 2016. DNA barcoding uncovers cryptic diversity in 50% of deep-sea Antarctic polychaetes. Royal Society Open Science 3:160432. DOI: 10.1098/rsos.160432.

Chao, A. 1984. Nonparametric Estimation of the Number of Classes in a Population. Scandinavian Journal of Statistics 11(4): 265-270.

Chapman, A. S. A., V. Tunnicliffe, and A. E. Bates. 2018. Both rare and common species make unique contributions to functional diversity in an ecosystem unaffected by human activities. Diversity and Distributions:1–11. DOI: 10.1111/ddi.12712.

Christiansen, B., A. Denda, and S. Christiansen. 2019. Potential effects of deep seabed mining on pelagic and benthopelagic biota. Marine Policy, in press. DOI: 10.1016/j.marpol.2019.02.014.

Clare, E. L., F. J. J. Chain, J. E. Littlefair, and M. E. Cristescu. 2016. The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data1. Genome 59:981–990.

Clarke, L. J., J. M. Beard, K. M. Swadling, and B. E. Deagle. 2017. Effect of marker choice and thermal cycling protocol on zooplankton DNA metabarcoding studies. Ecology and Evolution 7:873–883. DOI: 10.1002/ece3.2667.

Convention on Biological Diversity, 29 December 1993, available at: https://www.cbd.int/convention/text/

Darby, B. J., T. C. Todd, and M. A. Herman. 2013. High-throughput amplicon sequencing of rRNA genes requires a copy number correction to accurately reflect the effects of management practices on soil nematode community structure. Molecular Ecology 22:5456–5471. DOI: 10.1111/mec.12480.

Dauvin, J. C. 2018. Twenty years of application of Polychaete/Amphipod ratios to assess diverse human pressures in estuarine and coastal marine environments: A review. Ecological Indicators 95:427–435. DOI: 10.1016/j.ecolind.2018.07.049.

De Smet, B., E. Pape, T. Riehl, P. Bonifácio, L. Colson, and A. Vanreusel. 2017. The community structure of deep-sea macrofauna associated with polymetallic nodules in the eastern part of the Clarion-Clipperton Fracture Zone. Frontiers in Marine Science 4:103. DOI: 10.3389/fmars.2017.00103.

Durden, J. M., L. E. Lallier, K. Murphy, A. Jaeckel, K. Gjerde, and D. O. B. Jones. 2018. Environmental Impact Assessment process for deep-sea mining in 'the Area.' Marine Policy 87:194–202. DOI: 10.1016/j.marpol.2017.10.013.

Elbrecht, V., and F. Leese. 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. PLoS ONE 10:e0130324. DOI: 10.1371/journal.pone.0130324.

Elbrecht, V., E. E. Vamos, K. Meissner, J. Aroviita, and F. Leese. 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods in Ecology and Evolution 8:1265–1275. DOI: 10.1111/2041-210X.12789.

Esling, P., F. Lejzerowicz, and J.Pawlowski. 2015. Accurate multiplexing and filtering for high-throughput amplicon-sequencing. Nucleic acids research 43(5): 2513-2524.

Evans, N. T., B. P. Olds, M. A. Renshaw, C. R. Turner, Y. Li, C. L. Jerde, A. R. Mahon, M. E. Pfrender, G. A. Lamberti, and D. M. Lodge. 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources 16:29–41.

Fonseca V.G., and D. Lallias. 2016. Metabarcoding Marine Sediments: Preparation of Amplicon Libraries. In: Bourlat S. (eds) Marine Genomics. Methods in Molecular Biology 1452. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3774-5_12.

Franzosa, E. A., T. Hsu, A. Sirota-Madi, A. Shafquat, G. Abu-Ali, X. C. Morgan, and C. Huttenhower. 2015. Sequencing and beyond: Integrating molecular "omics" for microbial community profiling. Nature Reviews Microbiology 13:360–372.

Fukushima T. and M. Nishijima. 2017. Taxonomic Problems in Environmental Impact Assessment (EIA) Linked to Ocean Mining and Possibility of New Technology Developments. In: Sharma R. (eds) Deep-Sea Mining. Springer, Cham. https://doi.org/10.1007/978-3-319-52557-0_16.

Garber, A. M., and C. E. Phelps. 1997. Economic foundations of cost-effectiveness analysis. Journal of Health Economics 16:1–31. DOI: 10.1016/S0167-6296(96)00506-1.

Gimmler, A., R. Korn, C. De Vargas, S. Audic, and T. Stoeck. 2016. The Tara Oceans voyage reveals global diversity and distribution patterns of marine planktonic ciliates. Scientific Reports 6:335555. DOI: 10.1038/srep33555.

Glover, A. G., T. G. Dahlgren, H. Wiklund, I. Mohrbeck, and C. R. Smith. 2016. An end-to-end DNA taxonomy methodology for benthic biodiversity survey in the Clarion-Clipperton Zone, central Pacific abyss. Journal of Marine Science and Engineering 4. DOI: 10.3390/jmse4010002.

Goineau, A., and A. J. Gooday. 2017. Novel benthic foraminifera are abundant and diverse in an area of the abyssal equatorial Pacific licensed for polymetallic nodule exploration. Scientific Reports 7:45288. DOI: 10.1038/srep45288.

Gollner, S., S. Kaiser, L. Menzel, D. O. B. Jones, A. Brown, N. C. Mestre, D. van Oevelen, L. Menot, A. Colaço, M. Canals, D. Cuvelier, J. M. Durden, A. Gebruk, G. A. Egho, M. Haeckel, Y. Marcon, L. Mevenkamp, T. Morato, C. K. Pham, A. Purser, A. Sanchez-Vidal, A. Vanreusel, A. Vink, and P. Martinez Arbizu. 2017. Resilience of benthic deep-sea fauna to mining activities. Marine Environmental Research 129:76–101. DOI: 10.1016/j.marenvres.2017.04.010.

Golovan, O. A., M. V. Malyutina, and A. Brandt. 2018. First record of the deep-sea isopod family Dendrotionidae (Isopoda: Asellota) from the Northwest Pacific with description of

two new species of Dendromunna. Marine Biodiversity 48:531–544. DOI: 10.1007/s12526-017-0725-0.

Gooday, A. J., and A. Goineau. 2019. The contribution of fine sieve fractions (63-150 μm) to foraminiferal abundance and diversity in an area of the eastern Pacific Ocean licensed for polymetallic nodule exploration. Frontiers in Marine Science 6. DOI: 10.3389/fmars.2019.00114.

Gooday, A. J., A. Goineau, and I. Voltski. 2015. Abyssal foraminifera attached to polymetallic nodules from the eastern Clarion Clipperton Fracture Zone: a preliminary description and comparison with North Atlantic dropstone assemblages. Marine Biodiversity 45:391–412. DOI: 10.1007/s12526-014-0301-9.

Groffman, P. M., J. S. Baron, T. Blett, A. J. Gold, I. Goodman, L. H. Gunderson, B. M. Levinson, M. A. Palmer, H. W. Paerl, G. D. Peterson, N. L. R. Poff, D. W. Rejeski, J. F. Reynolds, M. G. Turner, K. C. Weathers, and J. Wiens. 2006. Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems 9:1–13. DOI: 10.1007/s10021-003-0142-z.

Halpern, B. S., H. M. Regan, H. P. Possingham, and M. A. McCarthy. 2006. Accounting for uncertainty in marine reserve design. Ecology Letters 9:2–11.

Hauquier, F., L. Macheriotou, T. N. Bezerra, G. Egho, P. Martínez Arbizu, and A. Vanreusel. 2018. Geographic distribution of free-living marine nematodes in the Clarion-Clipperton Zone: implications for future deep-sea mining scenarios. Biogeosciences Discussions. DOI: 10.5194/bg-2018-492.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270:313–321. DOI: 10.1098/rspb.2002.2218.

Hein, J. R., and S. Petersen. 2013. The geology of manganese nodules. Pages 7–13 in E. Baker and Y. Beaudoin, editors. Manganese Nodules: A physical, biological, environmental, and technical review. Secretariat of the Pacific Community.

International Seabed Authority. 2019. Draft regulations on exploitation of mineral resources in the Area. ISBA/25/C/WP.1. Kingston, Jamaica.

International Seabed Authority. 2018. Draft Regulations on Exploitation of Mineral Resources in the Area ISBA/25/C/.

International Seabed Authority. 2017. A Discussion Paper on the development and drafting of Regulations on Exploitation for Mineral Resources in the Area (Environmental Matters). Kingston, Jamaica.

Janssen, A., S. Kaiser, K. Meißner, N. Brenke, L. Menot, and P. M. Arbizu. 2015. A reverse taxonomic approach to assess macrofaunal distribution patterns in abyssal pacific polymetallic nodule fields. PLoS ONE 10:e0117790. DOI: 10.1371/journal.pone.0117790.

Johnson, S. B., A. Warén, V. Tunnicliffe, C. Van Dover, C. G. Wheat, T. F. Schultz, and R. C. Vrijenhoek. 2015. Molecular taxonomy and naming of five cryptic species of Alviniconcha snails (Gastropoda: Abyssochrysoidea) from hydrothermal vents. Systematics and Biodiversity 13:278–295. DOI: 10.1080/14772000.2014.970673.

Jones, D. O. B., S. Kaiser, A. K. Sweetman, C. R. Smith, L. Menot, A. Vink, D. Trueblood, J. Greinert, D. S. M. Billett, P. M. Arbizu, T. Radziejewska, R. Singh, B. Ingole, T. Stratmann, E. Simon-Lledó, J. M. Durden, and M. R. Clark. 2017. Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. PLoS ONE 12:e0171750. DOI: 10.1371/journal.pone.0171750.

Kosakyan, A., M. Mulot, E.A.D. Mitchell, and E. Lara. 2015. Environmental DNA COI barcoding for quantitative analysis of protists communities: A test using the Nebela collaris complex (Amoebozoa; Arcellinida; Hyalospheniidae). European journal of protistology 51(4): 311-320.s

Kozich, J.J., S.L. Westcott, N.T. Baxter, S.K. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology AEM-01043.

Lambshead, P. J. D., C. J. Brown, T. J. Ferrero, L. E. Hawkins, C. R. Smith, and N. J. Mitchell. 2003. Biodiversity of nematode assemblages from the region of the Clarion-Clipperton Fracture Zone, an area of commercial mining interest. BMC Ecology 3:1–12. DOI: 10.1186/1472-6785-3-1.

Laroche, O., S. A. Wood, L. A. Tremblay, J. I. Ellis, G. Lear, and X. Pochon. 2018. A cross-taxa study using environmental DNA/RNA metabarcoding to measure biological impacts of offshore oil and gas drilling and production operations. Marine Pollution Bulletin 127:97–107. DOI: 10.1016/j.marpolbul.2017.11.042.

Le, J.T., L.A. Levin, and R.T. Carson. 2017. Incorporating ecosystem services into environmental management of deep-seabed mining. Deep-Sea Research II. 137:486-503. http://dx.doi.org/10.1016/j.dsr2.2016.08.007.

Leduc, D., S. D. Nodder, K. Berkenbusch, and A. A. Rowden. 2015. Effect of core surface area and sediment depth on estimates of deep-sea nematode genus richness and community structure. Marine Biodiversity 45:349–356. DOI: 10.1007/s12526-014-0271-y.

Lejzerowicz, F., P. Esling, and J. Pawlowski. 2014. Patchiness of deep-sea benthic Foraminifera across the Southern Ocean: Insights from high-throughput DNA sequencing. Deep-Sea Research Part II 108:17–26. http://dx.doi.org/10.1016/j.dsr2.2014.07.018

Lejzerowicz, F., P. Esling, L. Pillet, T. A. Wilding, K. D. Black, and J. Pawlowski. 2015. High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. Scientific Reports 5:13932. DOI: 10.1038/srep13932.

Leray, M., J.Y. Yang, C.P. Meyer, S.C. Mills, N. Agudelo, V. Ranwez, J.T. Boehm, and R.J. Machida. 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in zoology 10(1): 34.

Leray, M., and N. Knowlton. 2017. Random sampling causes the low reproducibility of rare eukaryotic OTUs in Illumina COI metabarcoding. PeerJ 5:e3006. DOI: 10.7717/peerj.3006.

Levin, L. A., K. Mengerink, K. M. Gjerde, A. A. Rowden, C. L. Van Dover, M. R. Clark, E. Ramirez-Llodra, B. Currie, C. R. Smith, K. N. Sato, N. Gallo, A. K. Sweetman, H. Lily, C. W. Armstrong, and J. Brider. 2016. Defining “serious harm” to the marine environment in the context of deep-seabed mining. Marine Policy 74:245–259. DOI: 10.1016/j.marpol.2016.09.032.

Lins, L., F. Leliaert, T. Riehl, S. P. Ramalho, E. A. Cordova, A. M. Esteves, and A. Vanreusel. 2016. Species variability and connectivity in the deep sea: evaluating effects of spatial heterogeneity and hydrodynamics. Biogeosciences Discussions 134. DOI: 10.5194/bg-2016-134.

Miljutin, D. M., M. A. Miljutina, P. M. Arbizu, and J. Galéron. 2011. Deep-sea nematode assemblage has not recovered 26 years after experimental mining of polymetallic nodules (Clarion-Clipperton Fracture Zone, Tropical Eastern Pacific). Deep-Sea Research Part I 58:885–897. DOI: 10.1016/j.dsr.2011.06.003.

Miljutina, M.A., D.M. Miljutin, R. Mahatma, et al. 2010. Marine Biodiversity 40:1. https://doi.org/10.1007/s12526-009-0029-0

Morard, R., M. Garet-Delmas, F. Mahé, S. Romac, J. Poulain, M. Kucera, and C. Vargas. 2018. Surface ocean metabarcoding confirms limited diversity in planktonic foraminifera but reveals unknown hyper-abundant lineages. Scientific Reports 8(1):2539.

Morton, J. T., C. Marotz, A. Washburne, J. Silverman, L. S. Zaramela, A. Edlund, K. Zengler, and R. Knight. 2019. Establishing microbial composition measurement standards with reference frames. Nature Communications 10.

National Deep Submergence Facility. 2018. Retrieved from http://www.whoi.edu/main/ndsf

Niner, H., J.A. Ardron, E.G. Escobar, M. Gianni, A. Jaeckel, D.O.B. Jones, L. Levin, C.R. Smith, T. Thiele, P.J. Turner, C.L. Van Dover, L. Watling, and K.M. Gjerde. 2018. Deep-sea mining with no net loss of biodiversity – An impossible aim. Frontiers in Marine Science 5:53. DOI: 10.3389/fmars.2018.00053.

O'donnell, J. L., R. P. Kelly, N. C. Lowell, and J. A. Port. 2016. Indexed PCR primers induce template- Specific bias in Large-Scale DNA sequencing studies. PLoS ONE 11:e0148698. DOI: 10.1371/journal.pone.0148698.

Pape, E., T. N. Bezerra, F. Hauquier, and A. Vanreusel. 2017. Limited spatial and temporal variability in meiofauna and nematode communities at distant but environmentally similar sites in an area of interest for deep-sea mining. Frontiers in Marine Science 4:205. DOI: 10.3389/fmars.2017.00205.

Pauvert, C., M. Buee, V. Laval, V. Edel-Hermann, L. Fauchery, A. Gautier, I. Lesur, J. Vallance, and C. Vacher. 2019. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. Fungal Ecology 41:23–33.

Porter, T. M., and M. Hajibabaei. 2018. Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. Molecular Ecology 27:313–338. DOI: 10.1111/mec.14478.

Quail, M. A., M. Smith, P. Coupland, T. D. Otto, S. R. Harris, T. R. Connor, A. Bertoni, H. P. Swerdlow, and Y. Gu. 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genomics 13:341. DOI: 10.1186/1471-2164-13-341.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., and M. Vecchione. 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences 7(9):2851-2899. DOI: 10.5194/bg-7-2851-2010.

Rio Declaration. 1992. A/CONF.151/26. Available online at: http://www.unesco.org/education/pdf/RIO_E.PDF

Rivera-Pinto, J., J. J. Egozcue, V. Pawlowsky-Glahn, R. Paredes, M. Noguera-Julian, and M. L. Calle. 2018. Balances: a new perspective for microbiome analysis. mSystems 3(4): e00053-18.

Rosli, N., D. Leduc, A. A. Rowden, and P. K. Probert. 2018. Review of recent trends in ecological studies of deep-sea meiofauna, with focus on patterns and processes at small to regional spatial scales. Marine Biodiversity 48:13–34. DOI: 10.1007/s12526-017-0801-5.

Rouse, G. W., N. G. Wilson, J. I. Carvajal, and R. C. Vrijenhoek. 2016. New deep-sea species of Xenoturbella and the position of Xenacoelomorpha. Nature 530:94–97.

Salazar, G., and S. Sunagawa. 2017. Marine microbial diversity. Current Biology 27:R489–R494. DOI: 10.1038/nature16545.

Shulse, C. N., B. Maillot, C. R. Smith, and M. J. Church. 2017. Polymetallic nodules, sediments, and deep waters in the equatorial North Pacific exhibit highly diverse and distinct bacterial, archaeal, and microeukaryotic communities. Microbiology Open 6:e428. DOI: 10.1002/mbo3.428.

Siddig, A. A. H., A. M. Ellison, A. Ochs, C. Villar-Leeman, and M. K. Lau. 2016. How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in Ecological Indicators. Ecological Indicators 60:223–230. DOI: 10.1016/j.ecolind.2015.06.036.

Simon-Lledó, E., B. J. Bett, V. A. I. Huvenne, T. Schoening, N. M. A. Benoist, R. M. Jeffreys, J. M. Durden, and D. O. B. Jones. 2019. Megafaunal variation in the abyssal landscape of the Clarion Clipperton Zone. Progress in Oceanography 170:119–133. DOI: 10.1016/j.pocean.2018.11.003

Sinniger, F., J. Pawlowski, S. Harii, A. J. Gooday, H. Yamamoto, P. Chevaldonné, T. Cedhagen, G. Carvalho, and S. Creer. 2016. Worldwide Analysis of Sedimentary DNA Reveals Major Gaps in Taxonomic Knowledge of Deep-Sea Benthos. Frontiers in Marine Science 3:92. DOI: 10.3389/fmars.2016.00092.

Smith, C. R., J. Drazen, and S. L. Mincks. 2006. Deep-sea Biodiversity and Biogeography : Perspectives from the Abyss. Kingston, Jamaica.

Stein, E. D., M. C. Martinez, S. Stiles, P. E. Miller, and E. V. Zakharov. 2014. Is DNA barcoding actually cheaper and faster than traditional morphological methods: Results from a survey of freshwater bioassessment efforts in the United States? PLoS ONE 9:e95525. DOI: 10.1371/journal.pone.0095525.

Sunagawa, S., L. P. Coelho, S. Chaffron, et al. 2015. Structure and function of the global ocean microbiome. Science 348. DOI: 10.1126/science.1261359

Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21(8):2045–2050. https://doi.org/ 10.1111/j.1365-294X.2012.05470.x

Thurber, A.R., A.K. Sweetman.,B.E. Narayanaswamy, D.O.B. Jones, J. Ingels, and R.L. Hansman. 2014. Ecosystem function and services provided by the deep sea. Biogeoscience Discussion. 11:3941–3963. http://dx.doi.org/10.5194/bgd-11-3941-2014.

Tremblay, J., K. Singh, A. Fern, E. S. Kirton, S. He, T. Woyke, J. Lee, F. Chen, J. L. Dangl, and S. G. Tringe. 2015. Primer and platform effects on 16S rRNA tag sequencing. Frontiers in Microbiology 6:771. DOI: 10.3389/fmicb.2015.00771.

Tunnicliffe, V., A. Metaxas, J. Le, E. Ramirez-Llodra, and L.A. Levin. Strategic environmental goals and objectives: setting the basis for environmental regulation of deep seabed mining. Marine Policy, in press. https://doi.org/10.1016/j.marpol.2018.11.010.

Turner, P.J., A.D. Thaler, A. Freitag, and P.C. Collins. 2019. Deep-sea hydrothermal vent ecosystem principles: Identification of ecosystem processes, services and communication of value. Marine Policy 101:118-124. https://doi.org/10.1016/j.marpol.2019.01.003.

United Nations General Assembly, Convention on the Law of the Sea, Article 145, 10 December 1982, available at: http://www.refworld.org/docid/3dd8fd1b4.html

Van Dover, C.L. 2014. Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: A review. Marine Environmental Research 102: 59-72. https://doi.org/10.1016/j.marenvres.2014.03.008.

Vanreusel, A., A. Hilario, P. A. Ribeiro, L. Menot, and P. M. Arbizu. 2016. Threatened by mining, polymetallic nodules are required to preserve abyssal epifauna. Scientific Reports 6:26808. DOI: 10.1038/srep26808.

Veillette, J., Sarrazin, J., Gooday, A.J., Galéron, J., Caprais, J.-C., Vangriesheim, A., Étoubleau, J., Christian, J.R., Juniper, S.K., 2007a. Ferromanganese nodule fauna in the Tropical North Pacific Ocean: species richness, faunal cover and spatial distribution. Deep-Sea Research I, 54: 1912-1935.

Veillette, J., S. K. Juniper, A. J. Gooday, and J. Sarrazin. 2007b. Influence of surface texture and microhabitat heterogeneity in structuring nodule faunal communities. Deep-Sea Research Part I 54:1936–1943. DOI: 10.1016/j.dsr.2007.06.012.

Wang, C. S., L. Liao, H. X. Xu, X. W. Xu, M. Wu, and L. Z. Zhu. 2010. Bacterial diversity in the sediment from polymetallic nodule fields of the Clarion-Clipperton Fracture Zone. Journal of Microbiology 48:573–585. DOI: 10.1007/s12275-010-0151-5.

Washburne, A. D., J. D. Silverman, J. T. Morton, D. J. Becker, D. Crowley, S. Mukherjee, L. A. David, and R. K. Plowright. 2019. Phylofactorization: a graph partitioning algorithm to identify phylogenetic scales of ecological data. Ecological Monographs 89:e01353.

Weaver P.P.E., D.S.M. Billett, and C.L. Van Dover. 2018. Environmental Risks of Deep-sea Mining. In: Salomon M., Markus T. (eds) Handbook on Marine Environment Protection. Springer, Cham. https://doi.org/10.1007/978-3-319-60156-4_11

Weymann, D., J. Laskin, R. Roscoe, K. A. Schrader, S. Chia, S. Yip, W. Y. Cheung, K. A. Gelmon, A. Karsan, D. J. Renouf, M. Marra, and D. A. Regier. 2017. The cost and cost

trajectory of whole-genome analysis guiding treatment of patients with advanced cancers. Molecular Genetics and Genomic Medicine 5:251–260. DOI: 10.1002/mgg3.281.

Wilson, G. D. F. 2017. Macrofauna abundance, species diversity and turnover at three sites in the Clipperton-Clarion Fracture Zone. Marine Biodiversity 47:323–347. DOI: 10.1007/s12526-016-0609-8.

Yasuhara, M., G. Hunt, T. M. Cronin, and H. Okahashi. 2009. Temporal latitudinal-gradient dynamics and tropical instability of deep-sea species diversity. Proceedings of the National Academy of Sciences of the United States of America 106:21717–21720. DOI: 10.1073/pnas.0910935106.

Yu, O. H., H. G. Lee, D. Kim, J. H. Wi, K. H. Kim, and C. M. Yoo. 2018. Characterization of Deep-sea Macrofauna in the Korean Exploration Claim Area in the Clarion-Clipperton Fracture Zone, Northeastern Pacific Ocean. Ocean Science Journal 53:301–314. DOI: 10.1007/s12601-018-0029-8.

Zaiko, A., A. Samuiloviene, A. Ardura, and E. Garcia-Vazquez. 2015. Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. Marine Pollution Bulletin 100:53–59. 10.1016/j.marpolbul.2015.09.030.

Zhang, G. K., F. J. J. Chain, C. L. Abbott, and M. E. Cristescu. 2018. Metabarcoding using multiplexed markers increases species detection in complex zooplankton communities. Evolutionary Applications 11:1901–1914. DOI: 10.1111/eva.12694.

Chapter 4: Using deep-sea imagery to examine ecosystem services associated with methane seeps

Jennifer T. Le, Peter Girguis, Lisa A. Levin

Abstract

Deep-sea imagery (pictures and videos) are routinely collected during at-sea expeditions and represent a large, often untapped repository of knowledge. At the same time, there is a pressing need for assessment of deep-sea ecosystem services in the face of human impact. We leveraged dive videos collected with the remotely-operated vehicle Hercules (operated by Ocean Exploration Trust), as well as biological trait analysis, in order to develop an approach that characterizes ecosystem services. Specifically, we focus on fisheries services and climate-regulating services related to carbon associated with methane seeps off the coast of southern California. Dive videos were used to qualitatively describe and compare megafaunal communities, ecological functions, and ecosystem services associated with three methane seeps and their surrounding areas: Point Dume (~725 m), Palos Verdes (~506 m), and Del Mar (~1023 m). A total of 100 megafaunal morphotypes were observed and represented seven functional groups: scavengers, benthic filter feeders and microcarnivores, benthic deposit feeders and bacterivores, demersal predators, pelagic predators, gelatinous plankton, and symbiont-bearing taxa. Our results suggest that the Del Mar seep and its adjacent areas provide the highest contributions to fisheries and carbon services. We also found that seep activity can have differential impacts on biological traits that contribute to ecosystem services. These findings show that deep-sea imagery can be used to make relative comparisons of ecosystem services at

and around methane seeps. Furthermore, our results have environmental management implications, such as identifying priorities for spatial protections. This study represents a first-step towards tapping into underutilized deep-sea imagery in order to characterize and quantify ecosystem services.

Introduction

The deep sea (here defined as greater than 200 m water depth) hosts diverse habitats that provide a myriad of ecosystem services (Armstrong et al., 2012; Thurber et al., 2014). Examples of provisioning services include fisheries landings for food (Clark et al., 2016) and genetic resources for industrial and pharmaceutical uses (Blasiak et al., 2019). There are regulating services such as carbon cycling (Cartapanis et al., 2016; Sweetman et al., 2019) and other element cycling (Blöthe et al., 2015; Huang et al., 2019). Deep-sea habitats also provide cultural services including education and outreach (Hoeberechts et al., 2015). An ecosystem services perspective directly links ecosystem structures and functions to human well-being (Millennium Ecosystem Assessment, 2005; Haines-Young & Potschin-Young, 2018), providing a tangible rationale for more holistic environmental management and protection (Le et al., 2017).

Ecosystem services can be difficult to quantify, especially in marine environments where interactions and boundaries can be dynamic and loosely-coupled (Barbier et al., 2011). However, technological developments have greatly aided deep-sea scientific research (e.g. Corinaldesi, 2015; Aguzzi et al., 2019). In particular, imaging (pictures and videos) of the deep sea provides useful information on physical and biological characteristics of underwater habitats (Macreadie et al., 2018). Imagery can be collected via underwater observatories (de Leo et al., 2018), drop cameras (Clayton & Dennison, 2017), landers (Lavaleye et al., 2018), autonomous underwater

vehicles (AUVs; Mejía-Mercado et al., 2019), remotely-operated vehicles (ROVs; Myhre et al., 2018), and human-occupied vehicles (HOVs; Gallo et al., 2015). Deep-sea expeditions routinely collect imagery for scientific (e.g. National Deep Submergence Facility, NEPTUNE Ocean Observatory), outreach (e.g. NOAA Okeanos Explorer, Ocean Exploration Trust, Schmidt Ocean Institute), and industry (e.g. Gates et al., 2017; Simon-Lledó et al., 2019) purposes. As a result, there is a wealth of imagery that continues to grow over time as interest in deep-sea exploration and resources expands.

Imagery has been instrumental to advances in our knowledge of deep-sea habitats and our ability to properly protect them. Amon et al. (2016) characterized the diversity and abundance of megafauna in a polymetallic nodule claim within the Clarion-Clipperton Fracture Zone, providing important baseline information for assessing impacts from potential mining. Another application of deep-sea imagery is evaluating vulnerable marine ecosystems, such as sponge gardens that enhance local biodiversity and impact biogeochemical cycling (Maldonado et al., 2016; Santín et al., 2018). Additionally, images and videos provide an opportunity to visualize organisms *in situ*, which can be important for behavioral observations (Katija et al., 2017) as well as for observing taxa that avoid nets (Ayma et al., 2016).

Application of deep-sea imagery to characterizing ecosystem services explicitly has been limited. Grupe et al. (2015) used ROV dive video to characterize the Del Mar methane seep and its megafaunal community. The authors found elevated densities of commercially valuable *Sebastolobus* spp. (thornyheads) at the seep relative to background areas. Other deep-sea studies that utilize imagery often discuss implications for ecosystem services (e.g. Chauvet et al., 2019; Cooper et al., 2019), but do not explicitly aim to do so. With the multitude of deep-sea imagery

being collected, there is opportunity to leverage existing data in order to characterize, and ideally quantify, ecosystem services.

In the summer of 2015, Ocean Exploration Trust (OET) completed an expedition to explore methane seeps and other deep-sea habitats along the southern California continental margin (USA) (Levin et al., 2016a). Methane seeps are found in every ocean from shallow to deep water depths (Judd, 2003) and are still being discovered today (Riedel et al., 2018; Seabrook et al., 2018). Geological processes lead to seepage of methane and sulfur-rich fluids from the seabed (Sibuet & Olu, 1997), which fuel chemoautotrophic microbial communities (Boetius et al., 2000; Orphan et al., 2002) that act as the base of a food web for distinct biological communities (Levin et al., 2005; Åström et al., 2018). Many "background" species can also be found at methane seeps (Levin et al., 2016b), aggregating around authigenic carbonates (Treude et al., 2011), snail egg towers (Levin & Dayton, 2009), or other structures that increase habitat heterogeneity. An additional layer of complexity exists along the northeastern Pacific continental margin in the form of an oxygen minimum zone (OMZ), which is a midwater feature of naturally-occurring low oxygen (> 22 μmol/kg, > 0.5 ml/l). The OMZ can intersect benthic environments to shape local biological communities (Sellanes et al., 2010; Gallo & Levin, 2016; Neira et al., 2019), and resulting ecosystem services, such as fish catch (Keller et al., 2015).

Fisheries species have been previously found at methane seeps. Examples include Tanner crabs on the Cascadia margin (Seabrook et al., 2019), and Patagonian toothfish in Chile (Sellanes et al., 2012). Southern California has four commercial deep-sea fisheries: shortspine thornyhead (*Sebastolobus altivelis*), longspine thornyhead (*Sebastolobus alascanus*), sablefish (*Anoplopoma fimbria*), and Dover sole (*Microstomus pacificus*) (Keller et al., 2015; Gallo et al., 2016). Several

of these species have previously been found on methane seeps (Grupe et al., 2015), but it is unclear how these habitats are utilized. Hypotheses include feeding in localized, high-productivity areas (Seabrook et al., 2019); breeding and laying eggs (Treude et al., 2011); avoiding predators (Tobler et al., 2016); or removing parasites (Tobler et al., 2007). These species also interact with seep environments through bioturbation (Yahel et al., 2008) and transporting chemosynthetic production to adjacent environments (Seabrook et al., 2019). As a result of these interactions, the Pacific Fishery Management Council considers methane seeps essential fish habitat (Pacific Fishery Management Council, 2019), which are all habitats necessary for fish feeding, growth, and reproduction.

Climate-regulating services related to carbon (hereafter referred to as "carbon services") may also be provided by methane seeps. Continental margins contribute disproportionately to global carbon and nutrient cycling (Elrod et al., 2004; Little et al., 2016): although they comprise approximately 20% of global ocean surface area (Jahnke et al., 2010), continental margins have been estimated to sequester more than 40% of carbon in the ocean (Muller-Karger et al., 2005). Additionally, the coupling of anaerobic oxidation of methane and sulfate reduction by seep microbes serves as a methane sink through carbonate precipitation (Naehr et al., 2007; Marlow et al., 2014). OMZs, like the one off the coast of southern California, can also contribute to regulating services through their influence on nitrogen and sulfur cycling (Gilly et al., 2013).

The objective of this paper is to develop an approach that characterizes deep-sea ecosystem services at and around methane seeps using deep-sea imagery. We adapt biological trait analysis to target ecosystem services (e.g. Rees et al., 2012) and focus specifically on fisheries and carbon services. These services are likely mediated, in part, by megafauna whereas services such as element cycling are facilitated by microbes which cannot be observed with

imagery. However, we do discuss visual indicators of microbially-driven services where relevant. We use examples from three southern California, upper slope, methane seeps (from north to south): Point Dume (724.5 m), Palos Verdes (505.6 m), and Del Mar (1023.4 m). For two of these (Point Dume and Palos Verdes), we provide the first detailed characterization of megafauna. Key questions addressed are: (1) Which megafaunal taxa are present at a given site? (2) What functional traits or behaviors do the community exhibit? (3) How might these traits promote ecosystem services? And (4) How can deep-sea exploration and observing be conducted in ways that facilitate quantification of ecosystem services? We examine the hypothesis that habitat heterogeneity at methane seeps provide more fisheries and carbon services than adjacent non-seep areas by testing for differences among active seep sites, transition areas, and non-seep background areas. We also hypothesize that fisheries and carbon services increase with diversity, which has been shown to increase ecological function, such as benthic fluxes of nutrients (Belley & Snelgrove, 2016), that can contribute to ecosystem services. Additionally, we investigate how these services relate to depth, dissolved oxygen concentrations, and temperature.

Methods

Study sites

The southern California continental margin is an active, narrow, steep slope, and is home to an expanding OMZ that sits between approximately 450-1100 m (Helly and Levin, 2004; Stramma et al., 2010; Bograd et al., 2015). Our three study sites (Figure 4.1) were chosen because they showed preliminary signs of both fisheries services (i.e. presence of commercial species) and carbon services (i.e. bacterial mat indicating primary production). They also included both “active seep” areas (characterized by visual indicators of bacterial mats, clam

beds, or bubbling) as well as "background" areas (no visual indicators of seepage). The Point Dume (mean depth 725 m) and Palos Verdes (mean depth 506 m) seeps were newly discovered during OET expedition NA066 (Levin et al., 2016a). The Point Dume seep lies along a submarine river channel within the core of the OMZ, peppered with carbonate chimneys that have visually evident fluid flow (Levin et al., 2016a). The Palos Verdes seep is less than 5 km from shore and characterized by large carbonate rocks covered by megafaunal aggregations (Levin et al., 2016a). The Del Mar seep (mean depth 1023 m) was discovered by graduate students at Scripps Institution of Oceanography in 2015 (Maloney et al., 2015) and has since been visited several times.

ROV dives

Exploratory dives were conducted by ROV Hercules in July and August 2015 as part of the OET southern California borderlands expedition NA066. High-definition video was taken continuously during each dive which ranged between 7-20 hours duration. The ROV recorded location, depth, temperature, conductivity, sound velocity, and oxygen concentrations. Because OET is focused on ocean exploration and telecommunication, we were not able to extract quantitative data from the dive videos due to changes in altitude, zoom, and non-visible laser references. However, qualitative descriptions based on presence-absence and frequency of occurrence are still useful, especially in deep-sea systems that are rarely visualized. Metadata from each dive are summarized in Table 4.1.

Video analysis

Video from each dive was segmented into five-minute clips that were each treated as a "sample" and annotated by-hand in MS Excel (the full protocol can be found in Appendix 4A). Information regarding the ROV setting (Table 4.2; Figure 4.2) and megafauna encountered (morphotype, location, behavior) was collected. Seep activity is separated into three categories: active seep sites with visual indicators of active seepage (e.g. dense bacterial mats and clam beds, bubbling), transition areas with visual indicators of sparse or prior seepage (e.g. patchy bacterial mats, dead clam beds, carbonates without signs of seepage), and non-seep background areas generally associated with soft sediment habitats. For the first minute of each video, animals were counted and identified to the highest possible taxonomic resolution. For the remaining four minutes, a full list of morphotypes was generated.

Ecosystem services trait analysis

Observable traits that support fisheries or carbon services were chosen (Table 4.3), and each morphotype was assigned a score for selected traits (Appendix 4B). We used fuzzy coding to capture the extent to which trait modalities contribute to each service (Chevenet et al., 1994). Each video was scored (both within the one-minute subset and the whole clip) for the morphotypes present that demonstrate the traits chosen. We were not able to calculate faunal densities from the videos due to unknown and variable camera field-of-view so we used presence-absence data. Scores were standardized by the number of morphotypes found in each clip. The minimum fisheries score a morphotype could have was zero whereas the maximum score was four. For carbon services, the minimum score was two and the maximum score was seventeen.

Statistical analysis

All statistical analyses were done in R (version 3.5.2.), using the base package unless otherwise noted. Data were tested for normality using a Shapiro-Wilk test. Because data did not meet normality conditions, non-parametric tests were used. The Kruskal-Wallis test-by-ranks was used to test for significant differences among groups (e.g. sites, seep activity, microhabitats), and a *post hoc* Dunn test with a Bonferroni correction (package 'dunn.test') was used to identify which groups were different. Correlations were tested using Spearman's rank coefficient. All ecosystem services score analyses were done for the first-minute subset as well as for the whole video clip in efforts to decrease temporal dependence among samples. Species accumulation curves were plotted using R package 'vegan'.

Results

Approximately 20,000 individuals from 100 morphotypes were identified in the videos and grouped into seven functional groups: scavengers, benthic filter feeders & microcarnivores, benthic deposit feeders & bacterivores, demersal predators, pelagic predators, gelatinous plankton, and symbiont-bearing taxa (Table 4.4; Figure 4.3). Demersal predators had the most morphotypes with 37, most of which were fish species (Figure 4.4A). Both pelagic predators and symbiont-bearing taxa had only three morphotypes included.

The most frequently occurring morphotypes among all sites were *Poralia rufescens* (jellyfish; 12.5%), *Sebastolobus altivelis* (shortspine thornyhead; 9.4%), *Voragonema pedunculata* (hydrozoan; 7.8%), Liponema anemones (6.9%), and *Nezumia liolepis* (smooth grenadier; 5.8%) (Table 4.5). There was a significant difference among the biological communities at our sites (ANOSIM, $R = 0.356$, $p < 0.01$) (package 'vegan'). Among sites, Palos

Verdes had the highest total number of morphotypes (79), followed by Del Mar (38) and lastly Point Dume (31). Species accumulation curves for each site show similar findings (Figure 4.5). Palos Verdes also had the highest number of morphotypes unique to the site (47) whereas Del Mar and Point Dume had fifteen and six unique morphotypes, respectively.

The first-minute subset was not representative of the whole clip relative to fisheries (X^2 = 47.16, df = 1, $p < 0.01$) and carbon scores (X^2 = 80.40, df = 1, $p < 0.01$), so results discussed are for the whole five-minute clip (scores for the one-minute subset are still shown in Table 4.6).

Point Dume (~698–757 m)

The Point Dume dive (H1456) spent approximately 37% of time at the active seep site, 25% in transition areas, and 37% in background areas (Table 4.2). This area had the lowest overlying oxygen concentrations with a mean of 2.76 μmol/kg (Table 4.1). During this dive, the most frequently occurring morphotypes were *P. rufescens* (18.5%), Liponema anemones (14.6%), galatheid crabs (11.7%), *S. altivelis* (8.2%), and *N. liolepis* (8.0%) (Table 4.5). These five morphotypes comprised over half of the megafauna occurrences during this dive. Other morphotypes were relatively rare: 22.5% of morphotypes only occurred once. Number of morphotypes was significantly negatively correlated with depth (ρ = -0.27, $p < 0.01$) and positively correlated with oxygen (ρ = 0.21, $p < 0.01$). Depth and oxygen negatively covaried with each other (ρ = -0.18, p = 0.01). There were no significant correlations ($p > 0.05$) between the number of functional groups in a video with depth, oxygen, or temperature.

Background areas had significantly higher fisheries scores than both active and transition areas by 20% and 16.5%, respectively (X^2 = 41.00, df = 2, $p < 0.01$; Table 4.6). Soft sediment substrates, which are associated with background areas, also had significantly higher fisheries

scores than bacterial mats by 16.3% ($X^2 = 41.62$, df = 1, $p < 0.01$). With respect to carbon, the same pattern was observed among seep activity: background areas had significantly higher scores than active and transition areas by 17.4% and 17.6%, respectively ($X^2 = 50.74$, df = 2, $p < 0.01$; Table 4.6). Oxygen negatively covaried with depth ($\rho = -0.18$, $p = 0.01$). Fisheries ($\rho = 0.27$, $p < 0.01$) and carbon scores ($\rho = 0.38$, $p < 0.01$) were significantly positively correlated with depth. Additionally, fisheries scores were significantly positively correlated with temperature at this site ($\rho = 0.17$, $p = 0.02$).

Palos Verdes (~278–799 m)

During the Palos Verdes dive (H1452), the ROV spent approximately 25% of its time at the active seep site, 1% in transition areas, and 66% in background areas (Table 4.2). The most frequently occurring morphotypes were *P. rufescens* (10.8%), *S. altivelis* (8.9%), *V. pedunculata* (6.4%), *N. liolepis* (5.4%), and a diversity of midwater fish (4.6%) (Table 4.5). The percentage of singletons, i.e. morphotypes that were observed exactly once, was 13.9%, which was lowest of all sites. Oxygen ($\rho = -0.97$, $p < 0.01$) and temperature ($\rho = -0.96$, $p < 0.01$) significantly covaried with depth. Number of morphotypes was significantly positively correlated with depth ($\rho = 0.60$, $p < 0.01$), and negatively correlated with oxygen ($\rho = -0.59$, $p < 0.01$) and temperature ($\rho = -0.58$, $p < 0.01$). The number of functional groups exhibited similar patterns with depth, oxygen, and temperature.

Palos Verdes transition areas, which included carbonate mounds, provided significantly higher fisheries scores than both active and background areas by 12.3% and 6.3%, respectively ($X^2 = 8.29$, df = 2, $p = 0.02$; Table 4.6). There were no significant differences among

microhabitats nor were there any differences in carbon scores. Neither fisheries nor carbon scores were significantly correlated with depth, oxygen, or temperature at this site.

Del Mar (~987–1030 m)

The Del Mar dive (H1444) spent approximately 60% of time at the active seep site and 40% in background areas (transition areas were not noted during this dive; Table 4.2). At the Del Mar seep, the most frequently occurring morphotypes were *V. pedunculata* (30.0%), *S. altivelis* (15.5%), *Eusergestes similis* (shrimp; 6.7%), a diversity of midwater fish (4.5%), and *Anoplopoma fimbria* (sablefish; 4.4%) and Lithodid crabs (4.4%) (Table 4.5). Neither the number of morphotypes nor functional groups were significantly correlated with depth, temperature, or oxygen.

There were no significant differences in fisheries or carbon scores among areas with different seep activity or microhabitats at Del Mar seep. However, fisheries scores were significantly negatively correlated with oxygen ($\rho = -0.40$, $p < 0.01$).

Across all three sites

Overall, fisheries and carbon scores were significantly positively correlated with each other ($\rho = 0.86$, $p < 0.01$), and the number of morphotypes observed (fisheries $\rho = 0.19$, $p < 0.01$; carbon $\rho = 0.18$, $p < 0.01$). However, neither score was correlated with the number of functional groups present nor the number of morphotypes present within any one functional group.

With respect to fisheries scores, Del Mar had significantly higher scores than Palos Verdes by 12% ($X^2 = 8.83$, df = 2, $p = 0.01$) (Figure 4.6A). Del Mar also had significantly higher

carbon scores than both Point Dume and Palos Verdes by 13.4% and 14%, respectively (X^2 = 15.03, df = 2, $p < 0.01$). Across all three sites, there were no significant differences in carbon scores among seep activity (i.e. active seep, transition, background) nor microhabitats (i.e. soft sediment, bacterial mat, clam bed, carbonate).

Overall, active seeps had significantly lower fisheries scores than background areas by 6% (X^2 = 14.02, df = 3, p = 0.01). Among active seeps, Del Mar had significantly higher fisheries (X^2 = 7.13, df = 2, p = 0.03) and carbon scores (X^2 = 12.35, df = 2, $p < 0.01$) than Point Dume by 15% and 20%, respectively (Figure 4.6B). Palos Verdes transitions areas had higher fisheries services than Point Dume transition areas by 8% (X^2 = 4.02, df = 1, p = 0.04), but significantly lower fisheries and carbon scores than Point Dume and Del Mar among background areas (fisheries X^2 = 27.83, df = 2, $p < 0.01$; carbon X^2 = 33.46, df = 2, $p < 0.01$).

Discussion

Describing the biological community

Deep-sea imagery is often used to describe biological communities (e.g. Amon et al., 2016; Cooper et al., 2019). When pictures and videos are collected in a systematic way (e.g. steady transects with known surface area), faunal densities, distributions, and biomass estimates can be produced, and compared over space and time (Dunlop et al., 2015; Smith et al., 2019). Qualitative data can also be helpful. In our study, number of morphotypes increases with oxygenation among sites: the Palos Verdes dive had the highest number of morphotypes and had the highest mean overlying oxygen of 20.24 μmol/kg (Table 4.1). This could be an artefact of the larger distance and wider depth range (~278–799 m) covered by the dive. However, oxygen has been shown to influence biodiversity on Pacific continental margins with a strong threshold

effect (Sperling et al., 2016). This threshold effect has been shown to decrease diversity beginning at approximately 22 μmol/kg (Sperling et al., 2016), a higher oxygen concentration than the dive mean, but the highest number of morphotypes observed in a single video at Palos Verdes (> 20) was associated with oxygen levels ranging from 2.18-2.26 μmol/kg. Within the Palos Verdes dive, the number of morphotypes observed in each video was negatively correlated with oxygen ($\rho = -0.59$, $p < 0.01$). Number of morphotypes was also positively correlated with depth ($\rho = 0.60$, $p < 0.01$). In some cases, hypoxic conditions can exert selective pressure that increases specialization of taxa for increased diversity (Rogers, 2000). Gallo & Levin (2016) found diverse assemblages of fish in the Pacific, Atlantic, and Indian Oceans with physiological, morphological, and behavioral adaptations for life in OMZs. Additionally, increased biodiversity with water depth to peak from 2000-3000 m has been documented in several taxa (Rex, 1981), such as demersal fish in the northeast Atlantic (Mindel et al., 2016) and cnidarians, echinoderms, and gastropods in the Caribbean (Hernández-Ávila et al., 2018). Because oxygen and depth covaried ($\rho = -0.97$, $p < 0.01$), it is not possible to separate their effect on number of morphotypes during the Palos Verdes dive.

In contrast to Palos Verdes, Point Dume lies within the core of the California OMZ with mean oxygen levels of 2.76 μmol/kg. Here, number of morphotypes was significantly correlated with oxygen ($\rho = 0.21$, $p < 0.01$) and depth ($\rho = -0.27$, $p < 0.01$). Because the Point Dume seep is in suboxic water, further decreases in dissolved oxygen may surpass physiological tolerances of some taxa (Seibel, 2011; Wishner et al., 2018). This may provide some evidence for loss of available habitat and shifting faunal distribution due to climate change (Cheung et al., 2009; Deutsch et al., 2015). As deoxygenation continues to expand and intensify the OMZ (Bograd et al., 2008; Stramma & Schmidtko, 2019), animals that cannot tolerate low oxygen conditions will

lose available habitat while those that can will distribute accordingly (Netburn & Koslow, 2015). The decrease in number of morphotypes with depth observed in Point Dume videos ($\rho = -0.27$, $p < 0.01$) may be driven by the significant negative correlation between oxygen and depth ($\rho = -0.18$, $p = 0.01$). The correlation between number of morphotypes and depth here is the opposite of that observed during the Palos Verdes dive, highlighting differential effects of environmental parameters at different locations along the continental margin.

Traits that support fisheries and carbon services

All three sites had some visual evidence of bacterial mats during the dive (Figure 4.7), likely indicating microbial sulfide oxidation and possibly some methane oxidation. Sulfide-oxidizing microbes can detoxify sulfidic water (Lavik et al., 2009), which could facilitate occurrence of morphotypes that contribute to ecosystem services. Sulfide oxidation also consumes oxygen with active seep areas, consuming two orders of magnitude more oxygen than non-seep areas (Boetius & Wenzhöfer, 2013). However, seep influence on sediment macrofauna communities, on which megafauna could be feeding, seems to be limited (Levin et al., 2000; Demopolous et al., 2018). Only 25% of morphotypes occurred on the bacterial mat, most frequently Liponema anemones (13.4%), *P. rufescens* (11.6%), and galatheid crabs (10.2%). One morphotype of polychaete was found exclusively on bacterial mats with two occurrences.

Our results suggest that the active seep areas of Point Dume have lower fisheries and carbon scores than transition and background areas (Table 4.6). Intense seepage with hydrogen sulfide and exceptionally low oxygen may act synergistically to reduce the occurrence of functional traits that generate ecosystem services. During the Point Dume dive, we did not find significant correlations between ecosystem services scores and oxygen ($p > 0.05$), which ranged

from 2.01-4.73 μmol/kg. However, scores were significantly correlated with water depth which negatively covaried with oxygen ($\rho = -0.18$, $p = 0.01$). Unfortunately, separating these effects with our dataset is not possible.

Palos Verdes transition areas provided significantly higher fisheries services than active and background areas (Table 4.6). As mentioned before, this is likely driven by the large aggregations of fish found on carbonate rocks in transition areas (Figure 4.6). While the utility of these scores could be improved with faunal densities, they provide preliminary insight about what types of microhabitats and which environmental variables may be important to specific services at specific sites. Methane seeps have been recognized as essential fish habitat (Pacific Fishery Management Council, 2019) and this trait-based approach could provide additional evidence for spatial protections. The focus on ecosystem services provides a targeted effort that can help guide research and management priorities.

In the case of the Del Mar seep, Grupe et al. (2015) found higher densities of commercially-valuable species at the active seep than in adjacent, background areas. Our results somewhat contrast because we found no significant differences in ecosystem services scores among the active seep and background area during the Del Mar dive. This suggests that the Del Mar area, in general, contributes more to fisheries and carbon services than our other study sites. However, background areas could potentially be influenced by the seep through movement of chemicals and animals from the seep to adjacent areas (Levin et al., 2016b).

One drawback to using deep-sea imagery for trait-based ecosystem services assessment is the need for visual evidence. The traits in Table 4.3 are not exhaustive of characteristics that can contribute to fisheries or carbon services, but they were ascertainable from our dive videos. While deep-sea imagery may not be able to confirm regulating services, like metatranscriptomics

could (e.g. Lan et al., 2019), it does provide insight on animal behavior that can support ecosystem services. For example, midwater fish (e.g. myctophids, bristlemouths, barbeled dragonfish) would often be seen near the benthos and swimming into it (Figure 4.6), which has been previously observed (Cantwell, 2015). This could potentially represent an important benthic-pelagic interaction that contributes to carbon export.

Recommendations for future studies and best practices

Expensive and limited ship time can make it difficult to collect ideal video data. The need to juggle multiple scientific goals during each ROV dive makes it impractical to conduct quantitative visual transects, and to generate sufficient number of replicate transects. This can create downstream constraints for data analysis such as lack of quantitative measures of densities and difficulty standardizing observations. Best practices for collecting pictures and videos from deep-sea sampling instruments could be useful (e.g. SERPENT tutorial for ROV pilots). Random and independent sampling could facilitate data analysis. Replicates could also be useful, in which cabled observatories may be appropriate (e.g. Monterey Accelerated Research System). ROV and AUV transects should be conducted with consistent altitude, zoom, and speed, as well as with a scale for size (often in the form of lasers a fixed distance apart). The resulting data can then be used to calculate faunal densities and other diversity metrics (e.g. Amon et al., 2016; Simon-Lledó et al., 2019). A quantitative transect would also allow for comparison among locations and time periods (e.g. Rosen & Lauermann, 2016). Accurate maps of the seafloor before dive operations can help ensure best use of time (Raineault et al., 2012), but perhaps most important are the designation of detailed scientific goals and objectives prior to surveying.

Environmental measurements should be made in association with imagery being taken. Physical and chemical properties, such as temperature, oxygen, and hydrogen sulfide at seeps, are important parameters that help shape the biological communities (Levin et al., 2005). Porewater chemistry influences the sediment community (Gieskes et al., 2011), which can contribute to fisheries services (i.e. as prey of commercial species) and carbon services (i.e. as bioturbators). Scientific tools exist to assess water chemistry such as *in situ* mass spectrometers that can be mounted on ROVs and niskin bottles that can be used to sample water at discrete depths. These environmental properties can help explain differences in diversity and distribution, and provide insight on how communities may change with human impact such as climate change (Sperling et al., 2016).

As imaging technology continues to advance, the resolution of pictures and videos becomes increasingly helpful for post-analysis (Bodenmann et al., 2017; Dumke et al., 2018). Imagery should be analyzed consistently, which may mean cross-referencing protocols and morphotypes if more than one person is conducting the analysis. Human bias is inherent to current image analysis but can be minimized with training (Matabos et al., 2017). As more deep-sea imagery is analyzed and libraries are produced, there are possibilities to incorporate machine learning algorithms in collaboration with computer science and programming (Qin et al., 2015). Machine learning techniques could facilitate mining of existing deep-sea imagery data. They often sit in labs untouched, representing an underutilized source of knowledge. These pictures and videos provide an opportunity to generate knowledge for habitats that are rarely visualized and are data-limited. Imagery is also routinely collected for non-biological purposes, such as instrument deployment, which could be an additional source of data.

Environmental management implications

The approach developed in this study can serve as an environmental decision-making tool, such as in the designation of spatial protections, consideration of ecosystem service tradeoffs, and understanding of context-dependent roles of methane seeps. This analysis can identify areas of high ecosystem services provision, such as the Del Mar seep that had relatively high fisheries and carbon scores, which may be important for designating essential fish habitat or marine-protected areas (Lindegren et al., 2018). An ecosystem-services approach can also investigate tradeoffs that may need to be considered during the environmental decision-making process (Boulton et al., 2016). For example, if methane seeps provide differential ecosystem services, one prioritization metric for spatial protections could be weighted ecosystem services scores (e.g. Werner et al., 2014). Lastly, results from this paper advance our understanding of ecosystem services associated with methane seeps. They highlight the context-dependent role of methane seeps in providing fisheries and carbon services along environmental gradients. For example, while the combination of seepage and low oxygen seemed to suppress ecosystem services scores at Point Dume, the Palos Verdes and Del Mar ecosystem services seemed to benefit from at least some seep activity.

Conclusions

In addition to describing biological communities, deep-sea imagery can be amenable to characterizing ecosystem services. Although standardized sampling would increase the capacity for quantification and comparison of ecosystem services across space and time, this study highlights how existing dive videos and analysis tools can be leveraged to generate information on ecosystem services, such as fisheries and climate-regulating services related to carbon. A

service-based approach links ecosystem structures and ecological processes to human well-being, which can provide recommendations for environmental decision-making. This is increasingly important as the deep sea continues to be impacted by human activity such as fishing, waste disposal, and climate change (Armstrong et al., 2019). Mapping of ecosystem services is a popular method of identifying vulnerable areas in shallow waters (Burkhard et al., 2018), and could help with marine spatial planning in deep water when making decisions and creating priorities.

Acknowledgements

J. Le was supported by the Stout Foundation (20161390) and the UC San Diego Frontiers of Innovation Scholars Program (g001). L. Levin was supported by NSF Grant OCE 1634172. The authors would like to thank the E/V Nautilus crew, ROV Hercules and Argus pilots, and Ocean Exploration Trust for providing us with the opportunity to work at-sea and with the videos used in this study. Thank you also to Shuting Yang, Onyeweenu Ogene, Bryant Jew, Sarah Simons, Kayla Pfohlman, Drew Lira, and Ayinde Abanu for spending countless hours analyzing these ROV dive videos; Erin Navarro, Maryanne Beckwith, Haleigh Yang, and Dr. Natalya Gallo for their help and expertise in identifying animals; the UC San Diego Library providing for amazing cloud services to host our data; and the Program for Interdisciplinary Environmental Research at Scripps Institution of Oceanography for support and inspiration.

Chapter 4, in part, is in preparation for submission for publication. Le, J.T., Girguis, P., and L.A. Levin. Using deep-sea imagery to examine ecosystem services associated with methane seeps. The dissertation author was the primary investigator and author of this material.

Tables

Table 4.1. Remotely-operated vehicle Hercules metadata from Ocean Exploration Trust expedition NA066 off of the southern California borderlands.

Dive Number	**Date** (2015)	**Site**	**Latitude** (°N)	**Longitude** (°W)	**Average Water Depth** (m)	**Average Temperature** (°C)	**Average Oxygen Concentration** (µmol/kg)	**Hours of dive analyzed**
H1456	9 August	Point Dume	33.94343	118.84175	724.5	5.55	2.76	16.6
H1452	4-5 August	Palos Verdes	33.68481	118.36662	505.6	7.29	20.24	20.8
H1444	27-28 July	West Del Mar	32.90397	117.78251	1023.4	4.12	15.54	7.1

Table 4.2. Observation type, observation options, and percentage of time the ROV spent doing the activity or at the type of environment throughout each dive (accounting for 100% of its time). Observation options are mutually exclusive within each category.

Observation Type	**Observation Options**	**Point Dume** (%)	**Palos Verdes** (%)	**Del Mar** (%)
ROV activity	Stationary: Inactive	17.5	16.7	17.2
	Stationary: Pan/Focus	8.6	19.4	29.6
	Stationary: Sampling	11.6	12.5	19.1
	Mobile: Search	58.8	43.0	17.9
	Mobile: Transect	3.5	8.4	16.2
Seep activity	Active Site	37.2	24.9	59.5
	Transition	24.5	1.4	0
	Off-site	37.0	66.2	40.5
	Water column	1.3	7.5	0
Microhabitat	Soft sediment (background)	53.1	76.6	57.2
	Carbonate	0	6.5	16.4
	Bacterial mat: full	5.7	0	17.3
	Bacterial mat: patchy	41.2	4.2	0
	Clam bed: full	0	0	7.2
	Clam bed: scattered	0	12.7	1.9

Table 4.3. Traits and their modalities that contribute to fisheries and climate-regulating services related to carbon. Higher scores indicate modalities that contribute more to the respective service.

Ecosystem service	Trait	Modality	Reference
Fisheries – characteristic	Commercially valuable	Yes (1) No (0)	Koslow et al., 2000
Fisheries – trophic support	Predator Prey	Active (2) Passive (1) No (0) Yes (1) No (0)	Yang & Somero, 1993; Jacobsen & Vetter, 1996; Hattori et al., 2009; Dufault et al., 2009; Gallo et al., 2016
Carbon – fixation and cycling	Feeding mode	Autotrophic (5) Predator (4) Filter feeder (3) Deposit feeder (2) Scavenger (1)	Doering et al., 1986; Reinthaler et al., 2010; Wilmers et al., 2012; Atwood et al., 2015
Carbon – transport	Mobility	High (3) Medium (2) Low (1) None (0)	
Carbon – transport	Movement	Swim (1) Crawl (1) Burrow (1) Sessile (0)	
Carbon – transport	Bioturbation	High (3) Medium (2) Low (1) None (0)	Vardaro et al., 2009; Martinetto et al., 2016; Hou et al., 2017; Gogina et al., 2020
Carbon – storage	Diel vertical migration	Yes (1) No (0)	Hidaka et al., 2001; Hudson et al., 2014; Klevjer et al., 2016
Carbon – storage	Calcification	Yes (1) No (0)	
Carbon – storage	Body size	> 10 cm (3) 3-10 cm (2) < 3 cm (1)	

Table 4.4. Functional groups used, morphotypes included in them, average score assigned to the morphotypes in the functional group, and frequency of occurrence throughout each dive as percentages.

Functional Group	**Morphotypes included**	**Average fisheries score**	**Average carbon score**	**Point Dume** (%)	**Palos Verdes** (%)	**Del Mar** (%)
Scavengers	Hagfish, shrimp, amphipods	2.00	10.53	9.1	4.3	16.2
Benthic filter feeders & microcarnivores	Sea anemones, sea pens, corals, sponges	1.63	5.71	15.4	14.6	6.0
Benthic deposit feeders & bacterivores	Sea cucumbers, urchins, snails, brittle stars	1.32	8.05	0.3	8.5	6.0
Demersal predators	Demersal and benthic fish, crabs, sea stars	3.09	11.40	43.0	40.9	29.6
Pelagic predators	Midwater fish, squid, chaetognaths	2.91	10.85	3.7	4.8	5.1
Gelatinous plankton	Jellies, ctenophores, siphonophores	1.01	7.26	23.9	26.9	35.6
Symbiont-bearing taxa	Vesicomyid clams, lucinid clams, folliculinids	0.93	10.60	4.6	0	1.5

Table 4.5. Frequency of occurrence of each morphotype, presented as a percentage of total morphotype occurrences, for each dive as well as among all dives.

Morphotype	Point Dume	Palos Verdes	Del Mar	All dives
Alepocephalus tenebrosus	0	0	2	0.3
Anoplopoma fimbria	0	3.6	4.4	2.3
Bathyraja spinosissmia	0	0	0.4	0
Cataetyx rubirostris	0	0	0	0
Cladorhizidae	0	0	0.4	0
Coryphaenoides acrolepis	0.2	0	0	0.1
Embassichthys bathybius	0	0.1	0	0
Epatratus spp	2.6	0.8	4.2	1.9
Glyptocephalus zachirus	0	0.2	0.9	0.2
Liparidae	0	0.1	0	0
Lyopsetta exilis	0	0.1	0	0.1
Merluccius productus	0	0.5	0	0.3
Microstomas pacificus	4.6	4	0.7	3.8
Midwater fish	3.7	4.6	4.5	4.2
Nemichthyidae	0	0.8	0	0.4
Nettastomatidae	0	1.2	0	0.6
Nezumia liolepis	8	5.4	0.2	5.8
Ophiodon elongatus	0	0	0	0
Rajidae sp	0	0	0	0
Scyliorhinidae	5.2	1.4	0	2.7
Sebastes spp	0	0.5	0	0.2
Sebastolobus alascanus	0.2	0	0	0.1
Sebastolobus altivelis	8.2	8.9	15.5	9.4
Zoarcid	2.4	0.1	0	1

Table 4.5. Continued

Morphotype	Point Dume	Palos Verdes	Del Mar	All dives
Holothuroidea	0.1	3.6	0.9	1.9
White Sea Cucumber	0	0.1	0	0
Strongylocentrotus fragilis	0	3.9	0	1.9
Ophidiidae sp	0	0	0	0
Ophiurida sp 01	0.3	2.8	0	1.5
Asteroidea sp 01	0.1	4.1	0	2
Asteronyx sp	0	0.8	0	0.4
Brisingidae	0	0.4	0	0.2
Hippasteria sp 01	0	0.2	0	0.1
Gonatus sp	0	0.1	0	0
Octopus	0	0.6	0	0.3
Pteropod	0	0.1	0.5	0.1
Eusergestes similis	6.4	2.9	6.7	4.7
Galatheid sp	11.7	0.4	0	4.7
Lithodidae sp	0	0.7	4.4	0.9
Lithodidae sp 02	0	2.1	0	1
Pandalopsis sp	0.1	0	2.4	0.3
Peracarid sp 01	0.1	0	0	0
Sergestidae sp	0.1	0.5	2.7	0.6
Chaetognath	0	0	0	0
Amphipod	0	0	0.2	0
Lucinidae	4.6	0	0	1.8
Vesicomyidae	0	0	0.2	0
Alia snail	0.2	0	0	0.1
Buccinidae sp 01	0	0	0.9	0.1

Table 4.5. Continued

Morphotype	Point Dume	Palos Verdes	Del Mar	All dives
Gastropod sp 01	0	0.1	0	0
Gastropod sp 02	0.1	0	0	0
Paguroidea	0	0	0.4	0
Provanna	0	0	3.1	0.4
Heteropolypus sp	0	0.7	0	0.3
Zoanthid	0	0.1	0	0
Umbellula sp	0	1.6	0	0.8
Actinaria sp 01	0	0.3	0	0.2
Actiniidae sp 01	0	0.2	3.8	0.6
Actiniidae sp 02	0.8	0.5	1.1	0.7
Actiniidae sp 03	0	0.4	0	0.2
Bolocera sp	0	0.1	0	0.1
Liponema sp	14.6	2.5	0.2	6.9
Funiculina sp	0	1.7	0	0.8
Pennatulacea sp 01	0	0.1	0	0
Petalidium suspiriosum	0	0	0	0
Sessiliflorae sp	0	1.6	0	0.8
Scyphozoa sp 01	0	0.2	0	0.1
Scyphozoa sp 02	0	0.1	0	0
Aeginura	0	0.5	0	0.3
Atolla sp	0	0.1	0	0
Jelly03	0	0.6	0	0.3
Poralia rufescens	18.5	10.8	0.2	12.5
Spinophiura jolliveti	0	0	2.4	0.3
Voragonema pedunculata	2.5	6.4	30	7.8

Table 4.5. Continued

Morphotype	Point Dume	Palos Verdes	Del Mar	All dives
Dromalia alexandri	0	4	0	2
Siphonophore	2.4	4.1	2.4	3.2
Bolinopsis sp	0.1	1.4	0	0.7
Ctenophora sp 01	0.1	1.5	0	0.8
Ctenophora sp 02	0.3	0.5	0	0.4
Ctenophore sp 03	0	0.1	0	0.1
Lampocteis cruentiventer	0	0.5	0.7	0.3
Serpulid Polychaete	0	0.1	0	0.1
Flatworm01	0	0.2	0.5	0.2
Flatworm02	0	0.1	0	0
Polychaete01	1.8	0.3	0.2	0.8
Polychaete02	0	0	0.4	0
Polychaete03	0	0	0.2	0
Polychaete04	0	0	0.2	0
Polynoidae	0.5	0.9	0	0.6
Siboglinidae	0	0	0.2	0
Encrusting Sponge	0	0	0.5	0.1
Porifera sp 01	0.1	0	0	0
Porifera sp 02	0	0.1	0	0
Porifera sp 03	0	0.2	0	0.1
Sponge	0	0	0	0
Folliculinidae	0	0	1.1	0.1
Foram01	0	0.5	0	0.2
Foraminifera	0	0	0.5	0.1
Tunicate01	0	0.1	0	0

Table 4.6. Summary of ecosystem services scores, standardized by the number of morphotypes, for each site. Transition areas were not delimited for Del Mar. Significant differences across sites are noted with *a*, *b*, *c* (horizontally); significant differences within sites are noted with *x*, *y*, *z* (vertically).

Service	Point Dume	Palos Verdes	Del Mar	Overall
Fisheries: five-minute	2.71 ± 0.86^{ab}	2.61 ± 0.82^{a}	3.09 ± 1.38^{b}	2.72 ± 0.95
Fisheries: one-minute	2.22 ± 0.59	2.42 ± 0.77	2.30 ± 1.30	2.32 ± 0.79
Fisheries: active	$2.38 \pm 0.78^{a,x}$	$2.35 \pm 0.76^{ab,x}$	2.98 ± 1.45^{b}	2.58 ± 1.09^{x}
Fisheries: transition	$2.52 \pm 0.45^{a,x}$	$2.84 \pm 0.66^{b,y}$	NA	2.52 ± 0.45^{xy}
Fisheries: background	$3.18 \pm 0.97^{a,y}$	$2.59 \pm 0.86^{b,x}$	3.29 ± 1.23^{a}	2.82 ± 0.98^{y}
Carbon: five-minute	11.69 ± 3.09^{a}	11.59 ± 3.20^{a}	13.96 ± 5.47^{b}	11.99 ± 3.69
Carbon: one-minute	9.47 ± 1.71	10.53 ± 2.30	10.56 ± 3.51	10.08 ± 2.33
Carbon: active	$10.61 \pm 2.67^{a,x}$	11.63 ± 2.66	14.03 ± 5.93^{b}	11.94 ± 4.34
Carbon: transition	10.58 ± 1.54^{x}	11.75 ± 2.55	NA	10.58 ± 1.54
Carbon: background	$13.57 \pm 3.42^{a,y}$	11.53 ± 3.45^{b}	13.84 ± 4.68^{a}	12.34 ± 3.73

Figures

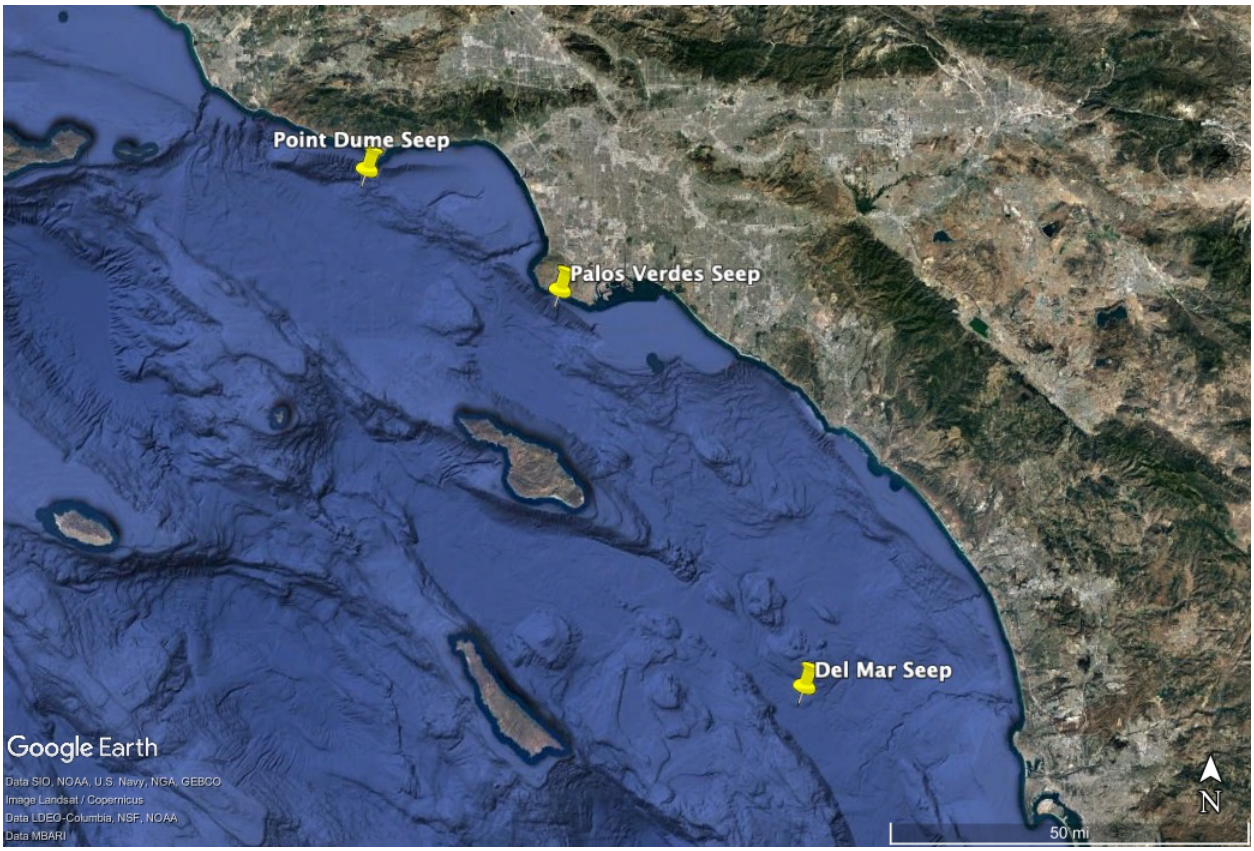

Figure 4.1. Geographic locations of our three methane seep study sites: Point Dume seep, Palos Verdes seep, and Del Mar seep.

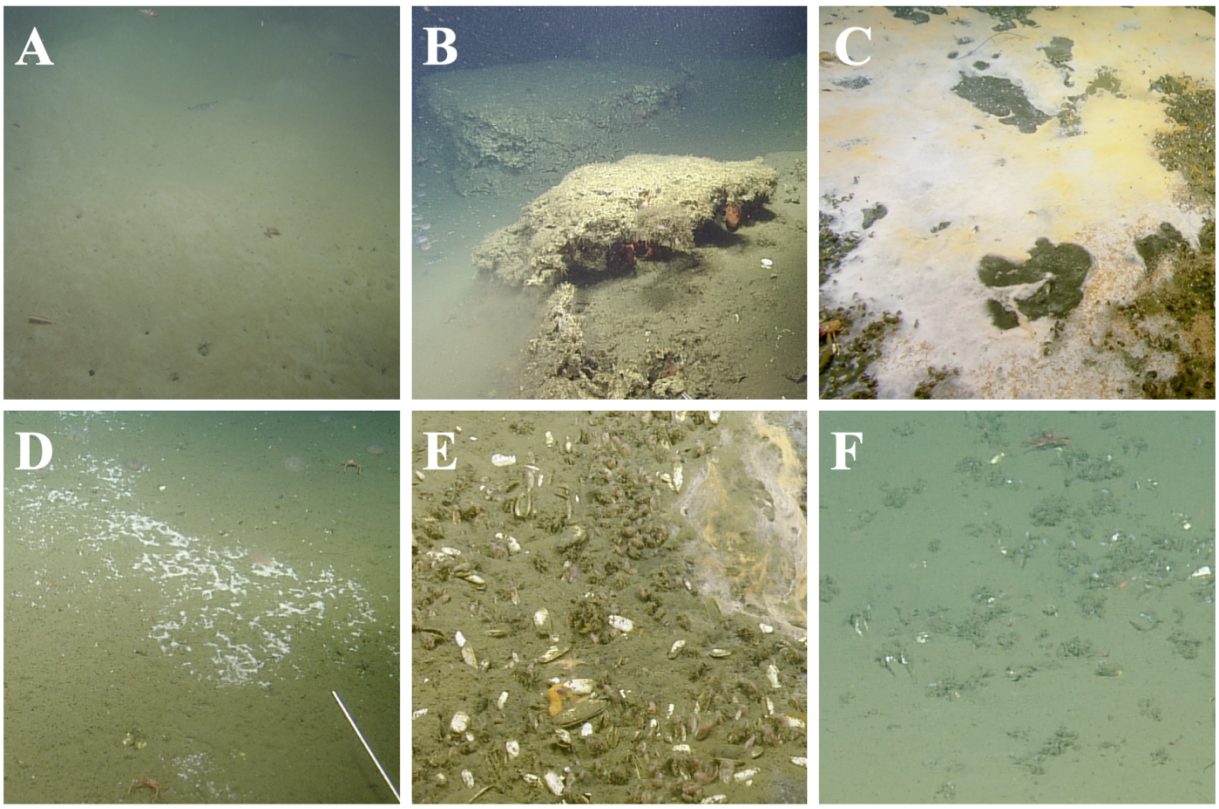

Figure 4.2. Examples of microhabitats observed during the dives: (A) soft sediment [background], (B) carbonate mounds, (C) full bacterial mat, (D) patchy bacterial mat, (E) full clam bed, and (F) scattered clam bed.

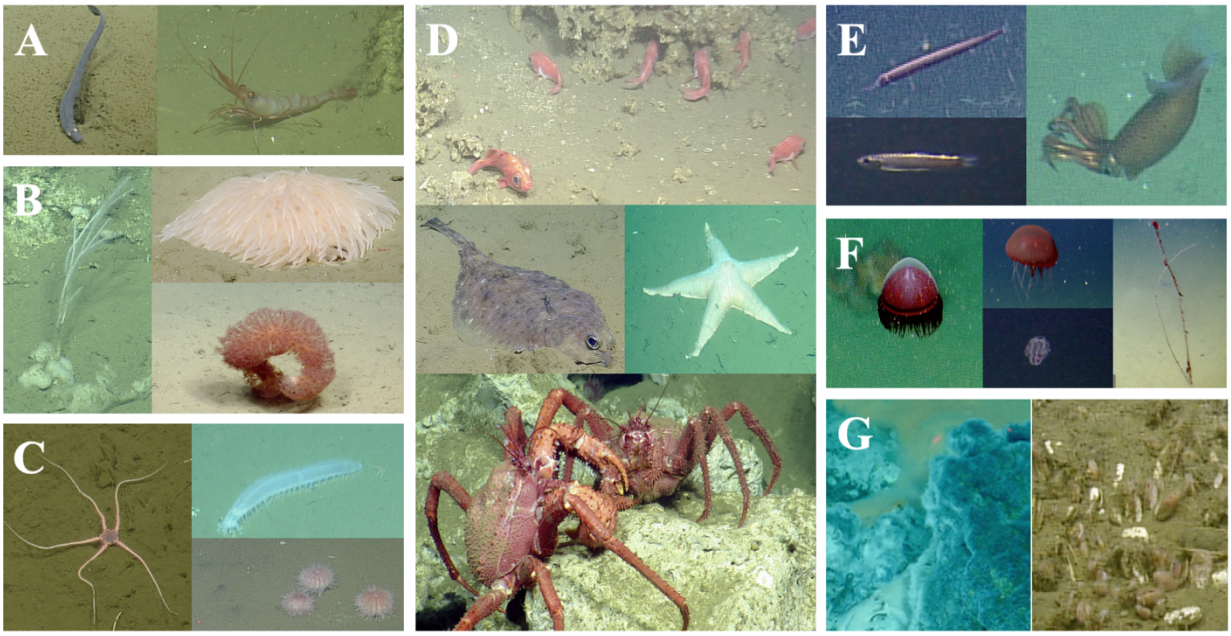

Figure 4.3. Example morphotypes of each functional group: (A) scavengers – hagfish, shrimp; (B) benthic filter feeders & microcarnivores – carnivorous sponge, sea anemone, sea pen; (C) benthic deposit feeders – brittle star, sea cucumber, sea urchin; (D) demersal predators – groundfish, sea stars, crabs; (E) pelagic predators – midwater fish, squid; (F) gelatinous plankton – jellies, ctenophores, siphonophores; and (G) symbiont-bearing taxa – folliculinid ciliates, vesicomyid clams.

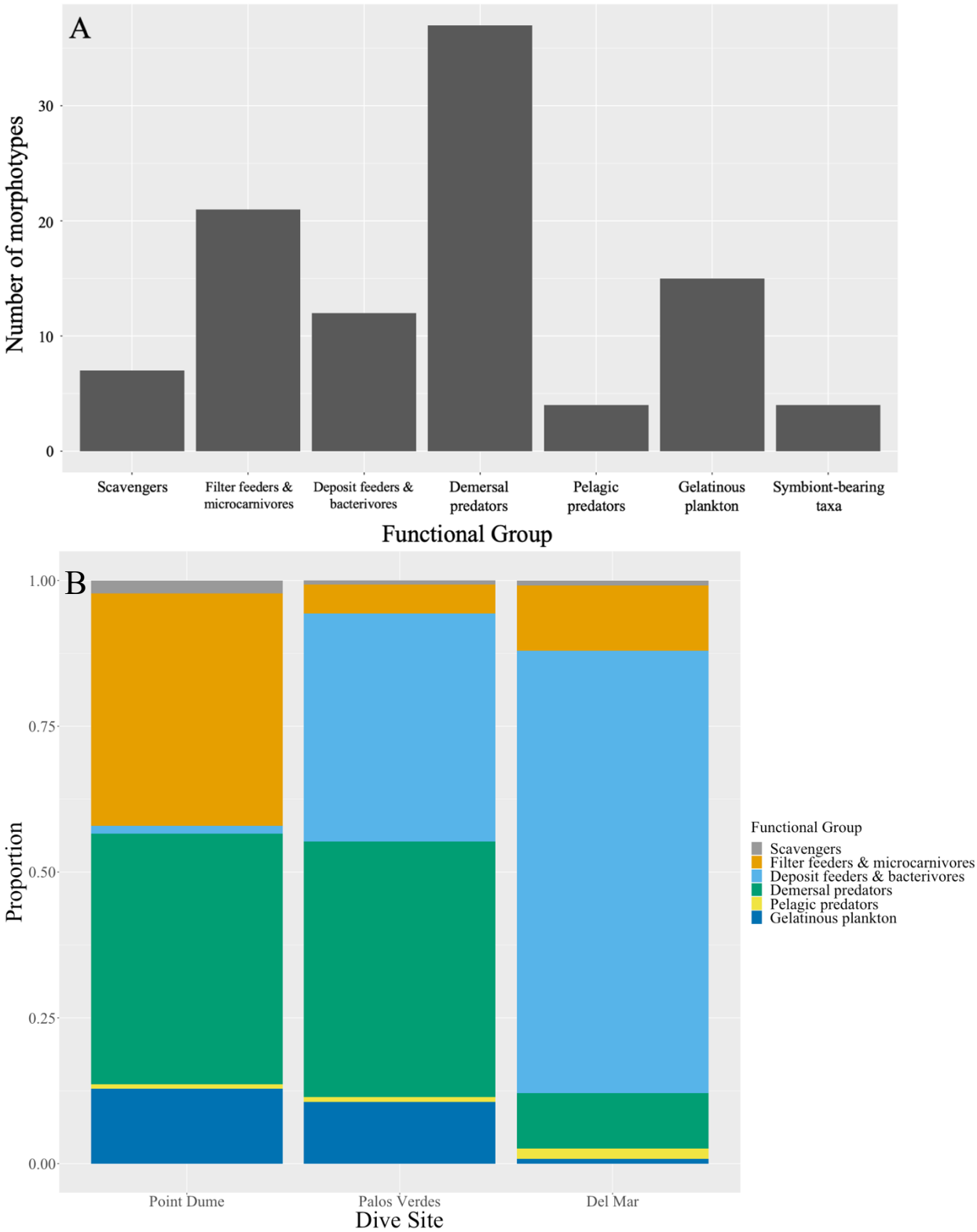

Figure 4.4. (A) The number of morphotypes included in each functional group, and (B) the relative abundance of each functional group at three methane seeps off southern California within the one-minute subset.

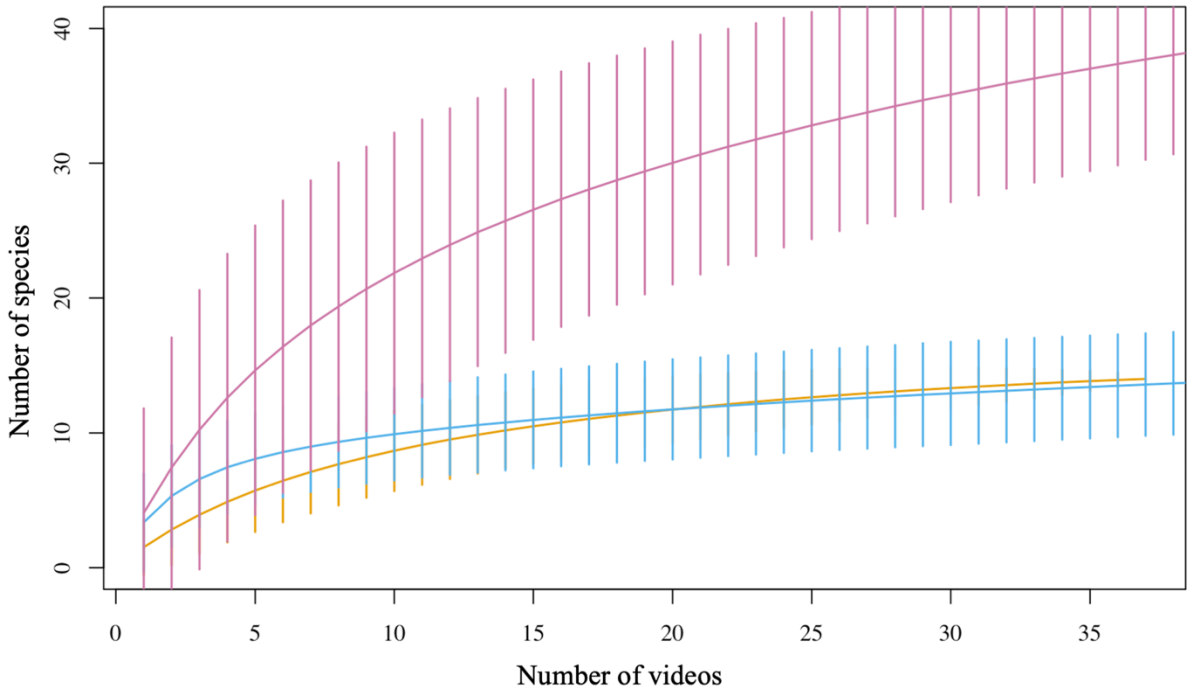

Figure 4.5. Species accumulation curves for the dives at Point Dume (blue), Palos Verdes (pink), and Del Mar (orange).

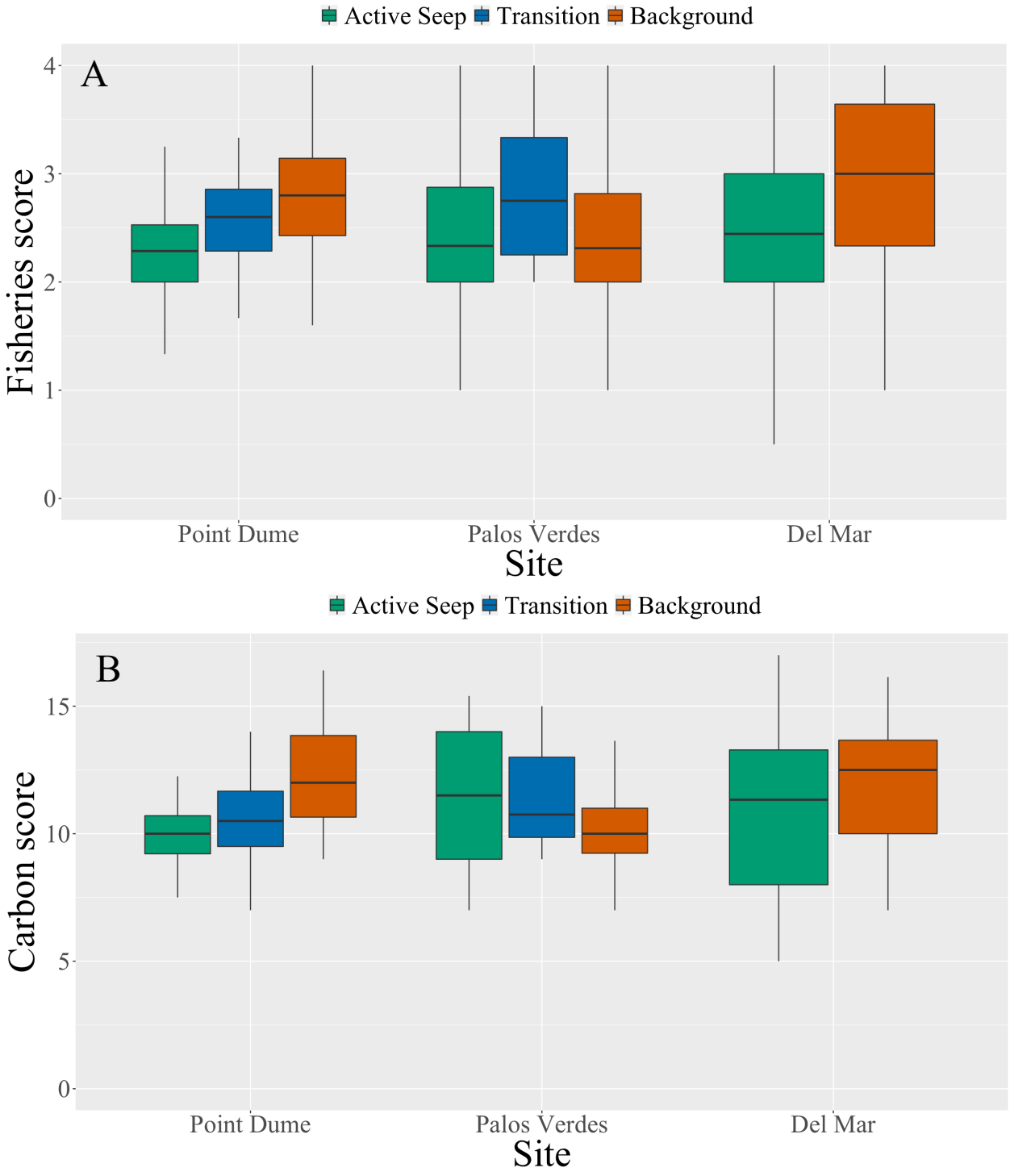

Figure 4.6. Boxplots showing quartiles of (A) fisheries and (B) carbon scores for each dive.

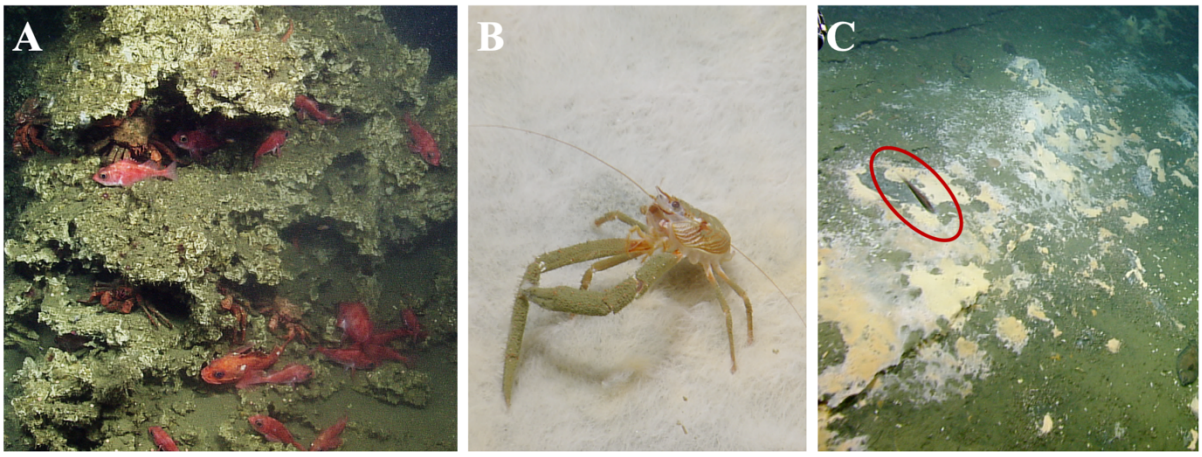

Figure 4.7. Examples of microhabitats and traits that support ecosystem services: (A) commercially-valuable rockfish and thornyhead spp. aggregating on carbonate rocks; (B) Galatheid crab feeding on bacterial mat; and (C) midwater fish observed at the active seep.

Appendices

Appendix 4A. Protocol used to analyze remotely-operated vehicle (ROV) dive videos in this study.

ROV: For each observation, fill out the video file name. Fill out the observation type: activity, habitat, substrate, lebenspurren (if applicable). Each file should have AT LEAST one of each of these observations. Then fill out the observation (e.g. stationary: inactive, soft sediment, etc.) and record the start and end times for each. Please use these characterizations unless something out of the ordinary comes up; then let Jen know. Note whether the observation is within the first minute of the video or not (Y/N).

1. Tag the video with an "activity" (what the ROV is doing) – indicate start and end times
    a. Stationary: Inactive
    b. Stationary: Pan/Focus (camera movement)
    c. Stationary: Sampling [Sampling type (push core, slurp, grab, Niskin)]
    d. Mobile: Search (exploratory)
    e. Mobile: Transect (directed movement)
2. Tag the video with a "habitat" as they appear in the video – indicate start and end times
    a. Active Site (seep, whale fall, canyon)
    b. Transition (some signs of activity, e.g. carbonate rocks but no bacterial mats)
    c. Off-site: Moving Towards
    d. Off-site: Moving Away
    e. Water Column (more than 3m off the bottom)
3. Tag the video with dominant "substrate" as they appear in the video – indicate start and end times
    a. Soft Sediment
    b. Carbonate (only really at seeps)
    c. Bacterial Mat: Full (more than 50% cover)
    d. Bacterial Mat: Patchy

e. Clam Bed: Full (more than 50% cover)
f. Clam Bed: Scattered
g. Mixed (more than one substrate visible other than sediment) – specify substrates in "notes" section
h. Make note of lebenspurren: lots of pits and burrows, ampharetids, etc.

FAUNA:

4. Working copy of fauna:
   https://docs.google.com/document/d/1nYeAXaQl79ZlkejMP42drUqg1jich8QDO7FS1UmLtlk/edit?usp=sharing
   Please check and update the document regularly. Name new fauna with an identifier (e.g. color), number, or both. Also be careful to avoid typos, which will make it difficult to sort later in the process: be consistent! Count individuals as they cross into the lower 2/3 of the screen.
5. Note the position of each individual.
   a. On Bottom (on top of the sediment, rock): On Top, Buried, Inside
   b. On Benthic Organism (on top of another organism that is attached to the bottom) – note what the benthic organism is
   c. Demersal (within one body length of the benthos)
   d. Water Column (more than one body length from the benthos)
6. Record what substrate each individual is on or hovering over ("location"), i.e. if a jelly is in the water column but hovering over bacterial mat, then tag this with bacterial mat. Use the same characterizations as Step 3.
7. Determine what each individual is doing.
   a. Stationary
   b. Mobile: Swimming (active), Drifting (inactive)
   c. Ventilating, Breathing
   d. Feeding
   e. Start a new line for any individual(s) that are doing different things, e.g. 5 anemones on the sediment, 2 anemones on stalked sponges
8. For high "density" areas (more than 25% of the frame), estimate the percent coverage of the organism, start time, and end time.
9. Also note any terrestrial plants, trash, etc. in the videos.
10. Miscellaneous: Make any notes about interesting observations, i.e. there were lots of/no particulates in the water, transition zone between brittle stars and holothurians, continuation of sampling from the previous video, etc.

Appendix 4B. Scores assigned to individual morphotypes found within this study.

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Alepocephalus tenebrosus	0	2	1	4	3	1	0	0	3	3	11	
Anoplopoma fimbria	1	2	1	4	3	3	0	0	3	4	13	
Alepocephalus tenebrosus	0	2	1	4	3	1	0	0	3	3	11	
Cataetyx rubirostris	0	2	0	4	3	3	0	0	3	2	13	
Cladorhizidae	0	1	1	3	0	0	0	0	2	2	5	Vacelet & Duport, 2004
Coryphaenoides acrolepis	0	2	1	4	3	3	0	0	3	3	13	
Embassichthys bathybius	0	2	1	4	3	3	0	0	3	3	13	
Epatratus spp	0	1	1	1	3	3	0	0	3	2	10	
Glyptocephalus zachirus	1	2	1	4	3	3	0	0	3	4	13	
Liparidae	0	2	1	4	3	3	0	0	2	3	12	

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Lyopsetta exilis	1	2	1	4	3	3	0	0	2	4	12	
Merluccius productus	1	2	1	4	3	3	0	0	3	4	13	
Microstomas pacificus	1	2	1	4	3	3	0	0	3	4	13	
Midwater fish	0	2	1	4	3	1	1	0	2	3	11	
Nemichthyidae	0	2	1	4	3	1	0	0	3	3	11	
Nettastomatidae	0	2	1	4	3	1	0	0	3	3	11	Merret, 1985
Nezumia liolepis	0	2	1	4	3	3	0	0	3	3	13	
Ophiodon elongatus	0	2	1	4	3	3	0	0	3	3	13	
Rajidae spp	0	2	1	4	3	3	0	0	3	3	13	
Scyliorhinidae	0	2	1	4	3	1	0	0	3	3	11	

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Sebastes spp	1	2	1	4	3	3	0	0	3	4	13	
Sebastolobus alascanus	1	2	1	4	3	3	0	0	3	4	13	
Sebastolobus altivelis	1	2	1	4	3	3	0	0	2	4	12	
Zoarcid	0	2	1	4	3	3	0	0	3	3	13	
Holothuroidea	0	0	1	2	1	3	0	0	2	1	8	Miller et al., 2000
White Sea Cucumber	0	0	1	2	1	3	0	0	2	1	8	Miller et al., 2000
Strongylocentrotus fragilis	0	1	1	2	1	3	0	1	2	2	9	
Ophidiidae sp	0	1	1	4	1	3	0	0	1	2	9	Pearson & Gage, 1984; Fujita & Ohto 1988
Ophiurida sp 01	0	1	1	4	1	3	0	0	1	2	9	Pearson & Gage, 1984; Fujita & Ohto 1988
Asteroidea sp 01	0	1	1	4	1	3	0	0	2	2	10	Lauerman, 1998

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Asteronyx sp	0	1	1	4	1	3	0	0	3	2	11	Pearson & Gage, 1984; Fujita & Ohto 1988
Brisingidae	0	1	1	3	1	3	0	0	3	2	10	Pearson & Gage, 1984; Fujita & Ohto 1988
Hippasteria sp 01	0	1	1	4	1	3	0	0	2	2	10	Lauerman, 1998
Gonatus sp	0	0	1	4	3	1	0	0	2	1	10	
Octopus sp	0	2	1	4	2	3	0	0	2	3	11	
Pteropod	0	0	1	3	2	0	1	0	1	1	7	Angel, 2000
Eusergestes similis	0	1	1	4	3	1	1	1	1	2	11	Cartes, 1993
Galatheid sp	0	1	1	2	1	3	0	1	2	2	9	Cartes, 1993
Lithodidae sp	0	1	1	2	1	3	0	1	3	2	10	Cartes, 1993
Lithodidae sp 02	0	1	1	2	1	3	0	1	3	2	10	Cartes, 1993

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Pandalopsis sp	0	1	1	4	2	1	1	1	1	2	10	Cartes, 1993
Peracarid sp 01	0	1	1	4	3	1	1	1	1	2	11	Cartes, 1993
Sergestidae sp	0	1	1	4	1	1	1	1	1	2	9	Flock & Hopkins, 1992
Chaetognath	0	0	1	4	2	0	1	0	1	1	8	Alvarez-Cadena, 1993
Amphipod	0	0	1	1	1	2	1	1	1	1	7	
Lucinidae	0	0	1	5	1	3	0	1	1	1	11	Peek, 1998
Vesicomyidae	0	0	1	5	1	3	0	1	1	1	11	Peek, 1998
Alia snail	0	0	1	2	1	2	0	1	1	1	7	
Buccinidae sp 01	0	0	1	2	1	2	0	1	1	1	7	
Gastropod sp 01	0	0	1	2	1	3	0	0	1	1	7	

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Gastropod sp 02	0	0	1	2	1	2	0	1	1	1	7	
Paguroidea	0	1	1	2	1	3	0	1	2	2	9	Cartes, 1993
Provanna	0	0	1	2	1	2	0	1	1	1	7	
Heteropolypus sp	0	0	1	3	0	0	0	1	2	1	6	
Zoanthid	0	0	1	3	0	0	0	1	1	1	5	
Umbellula sp	0	0	1	3	0	0	0	0	3	1	6	
Actinaria sp 01	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977
Actiniidae sp 01	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977
Actiniidae sp 02	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977
Actiniidae sp 03	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Bolocera sp	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977
Liponema sp	0	1	1	3	0	0	0	0	2	2	5	Purcell, 1977
Funiculina sp	0	0	1	3	0	0	0	0	3	1	6	Best, 1988
Pennatulacea sp 01	0	0	1	3	0	0	0	0	3	1	6	Best, 1988
Petalidium suspiriosum	0	1	1	4	2	1	1	1	1	2	10	Cartes, 1993
Sessiliflorae sp	0	0	1	3	0	0	0	0	3	1	6	Best, 1988
Scyphozoa sp 01	0	0	1	3	2	0	0	0	2	1	7	Angel, 1982; Larson, 1991
Scyphozoa sp 02	0	0	1	3	2	0	0	0	2	1	7	Angel, 1982; Larson, 1991
Aeginura	0	0	1	3	2	0	0	0	2	1	7	Angel, 1982; Larson, 1991
Atolla sp	0	0	1	3	2	0	0	0	2	1	7	Angel, 1982; Larson, 1991

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Jelly03	0	0	1	3	2	0	0	0	2	1	7	Larson, 1991
Poralia refescens	0	0	1	3	2	0	0	0	2	1	7	Larson, 1991
Spinophiura jolliveti	0	1	1	4	1	3	0	0	1	2	9	Pearson & Gage, 1984; Fujita & Ohto 1988
Voragonema peduncula ta	0	0	1	3	2	0	0	0	2	1	7	Larson, 1991
Dromalia alexandri	0	0	1	3	2	1	0	0	2	1	8	Hissmann, 2004
Siphonophore	0	0	1	3	2	0	1	0	3	1	9	
Bolinopsis sp	0	0	1	3	2	0	0	0	2	1	7	Mills, 1995; Angel, 1982
Ctenophora sp 01	0	0	1	3	2	0	0	0	2	1	7	Mills, 1995; Angel, 1982
Ctenophora sp 02	0	0	1	3	2	0	0	0	2	1	7	Mills, 1995; Angel, 1982
Ctenophore sp 03	0	0	1	3	2	0	0	0	2	1	7	Mills, 1995; Angel, 1982

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Lampocteis cruentiventer	0	0	1	3	2	0	0	0	2	1	7	Mills, 1995; Angel, 1982
Serpulid Polychaete	0	0	1	2	1	0	0	0	1	1	4	Bornhold & Milliman, 1973
Flatworm01	0	0	1	2	1	2	0	0	1	1	6	
Flatworm02	0	0	1	2	1	2	0	0	1	1	6	
Polychaete01	0	0	1	2	1	2	0	0	1	1	6	
Polychaete02	0	0	1	2	1	2	0	0	1	1	6	
Polychaete03	0	0	1	2	1	2	0	0	1	1	6	
Polychaete04	0	0	1	2	1	2	0	0	1	1	6	
Polynoidae	0	0	1	4	1	2	0	0	1	1	8	Chevaldonné, 1998
Siboglinidae	0	0	1	2	1	2	0	0	1	1	6	Hilário et al., 2011

Appendix 4B. Continued

Taxa	Commercial	Predator of target	Prey of target	Feeding mode	Mobility	Bioturbation	Diel vertical migration	Calcification	Body size	Sum_Fish	Sum_Carbon	Reference
Encrusting Sponge	0	0	1	3	0	0	0	0	3	1	6	
Porifera sp 01	0	0	1	3	0	0	0	0	3	1	6	
Porifera sp 02	0	0	1	3	0	0	0	0	3	1	6	
Porifera sp 03	0	0	1	3	0	0	0	0	3	1	6	
Sponge	0	0	1	3	0	0	0	0	3	1	6	
Folliculinidae	0	0	0	5	0	0	0	0	1	0	6	Pasulka et al., 2017
Foram01	0	0	0	2	0	2	0	1	1	0	6	
Foraminifera	0	0	0	2	0	2	0	1	1	0	6	
Tunicate 01	0	0	1	3	2	0	0	0	3	1	8	

References

Aguzzi, J., D. Chatzievangelou, S. Marini, E. Fanelli, R. Danovaro, S. Flögel, N. Lebris, F. Juanes, F. C. De Leo, J. Del Rio, L. Thomsen, C. Costa, G. Riccobene, C. Tamburini, D. Lefevre, C. Gojak, P. M. Poulain, P. Favali, A. Griffa, A. Purser, D. Cline, D. Edgington, J. Navarro, S. Stefanni, S. D'Hondt, I. G. Priede, R. Rountree, and J. B. Company. 2019. New High-Tech Flexible Networks for the Monitoring of Deep-Sea Ecosystems. Environmental Science and Technology 53:6616–6631.

Alvarez-Cadena, J. N. 1993. Feeding of the Chaetognath Sagitta elegans Verrill.

Amon, D. J., A. F. Ziegler, T. G. Dahlgren, A. G. Glover, A. Goineau, A. J. Gooday, H. Wiklund, and C. R. Smith. 2016. Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. Scientific Reports 6:30492.

Angel, M. V, P. Hargreaves, P. Kirkpatrick, and P. Domanski. 1982. Low variability in planktonic and micronektonic populations at 1,000m depth in the vicinity of 42 degree N, 17 degree W; Evidence against diel migratory behavior in the majority of species. Biological Oceanography 1:287–319.

Angel, M. V, P. Hargreaves, P. Kirkpatrick, and P. Domanski. 1982. Low variability in planktonic and micronektonic populations at 1,000m depth in the vicinity of 42 degree N, 17 degree W; Evidence against diel migratory behavior in the majority of species. Biological Oceanography 1:287–319.

Armstrong, C. W., N. S. Foley, R. Tinch, and S. van den Hove. 2012. Services from the deep: Steps towards valuation of deep sea goods and services. Ecosystem Services 2:2–13.

Armstrong, C. W., G. K. Vondolia, N. S. Foley, L. A. Henry, K. Needham, and A. M. Ressurreicao. 2019. Expert assessment of risks posed by climate change and anthropogenic activities to ecosystem services in the deep North Atlantic. Frontiers in Marine Science 6:158.

Åström, E. K. L., M. L. Carroll, W. G. Ambrose, A. Sen, A. Silyakova, and J. L. Carroll. 2018. Methane cold seeps as biological oases in the high-Arctic deep sea. Limnology and Oceanography 63:S209–S231.

Atwood, T. B., R. M. Connolly, E. G. Ritchie, C. E. Lovelock, M. R. Heithaus, G. C. Hays, J. W. Fourqurean, and P. I. Macreadie. 2015. Predators help protect carbon stocks in blue carbon ecosystems. Nature Climate Change 5:1038–1045.

Ayma, A., J. Aguzzi, M. Canals, G. Lastras, N. Bahamon, A. Mecho, and J. B. Company. 2016. Comparison between ROV video and Agassiz trawl methods for sampling deep water fauna of submarine canyons in the Northwestern Mediterranean Sea with observations on

behavioural reactions of target species. Deep-Sea Research Part I: Oceanographic Research Papers 114:149–159.

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, and Silliman BR. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs 81:169–193.

Belley, R., and P. V. R. Snelgrove. 2016. Relative contributions of biodiversity and environment to benthic ecosystem functioning. Frontiers in Marine Science 3:242.

Best, B. 1988. Passive Suspension Feeding in a Sea Pen: Effects of Ambient Flow on Volume Flow Rate and Filtering Efficiency. The Biological Bulletin 175:332–342.

Blasiak, R., J. B. Jouffray, C. C. C. Wabnitz, E. Sundström, and H. Osterblom. 2018. Corporate control and global governance of marine genetic resources. Science Advances 4.

Blöthe, M., A. Wegorzewski, C. Müller, F. Simon, T. Kuhn, and A. Schippers. 2015. Manganese-Cycling Microbial Communities Inside Deep-Sea Manganese Nodules. Environmental Science and Technology 49:7692–7700.

Bodenmann, A., B. Thornton, R. Nakajima, and T. Ura. 2017. Methods for quantitative studies of seafloor hydrothermal systems using 3D visual reconstructions. ROBOMECH Journal 4:22.

Boetius, A., K. Ravenschlag, C. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B. Barker Jorgensen, U. Witte, and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626.

Boetius, A., and F. Wenzhöfer. 2013. Seafloor oxygen consumption fuelled by methane from cold seeps. Nature Geoscience 6:725–734.

Bograd, S. J., M. P. Buil, E. Di Lorenzo, C. G. Castro, I. D. Schroeder, R. Goericke, C. R. Anderson, C. Benitez-Nelson, and F. A. Whitney. 2015. Changes in source waters to the Southern California Bight. Deep-Sea Research Part II: Topical Studies in Oceanography 112:42–52.

Bograd, S. J., C. G. Castro, E. Di Lorenzo, D. M. Palacios, H. Bailey, W. Gilly, and F. P. Chavez. 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. Geophysical Research Letters 35:1–6.

Bornhold, B., and J. Milliman. 1973. Generic and Environmental Control of Carbonate Mineralogy in Serpulid (Polychaete) Tubes. The Journal of Geology 81:363–373.

Boulton, A. J., J. Ekebom, and G. már Gíslason. 2016. Integrating ecosystem services into conservation strategies for freshwater and marine habitats: a review. Aquatic Conservation: Marine and Freshwater Ecosystems 26:963–985.

Burkhard, B., J. Maes, M. B. Potschin-Young, F. Santos-Martín, D. Geneletti, P. Stoev, L. Kopperoinen, C. M. Adamescu, B. Adem Esmail, I. Arany, A. Arnell, M. Balzan, D. N. Barton, P. Van Beukering, S. Bicking, P. A. V. Borges, B. Borisova, L. Braat, L. M. Brander, S. Bratanova-Doncheva, S. Broekx, C. Brown, C. Cazacu, N. Crossman, B. Czúcz, J. Daněk, R. de Groot, D. Depellegrin, P. Dimopoulos, N. Elvinger, M. Erhard, N. Fagerholm, J. Frélichová, A. Grêt-Regamey, M. Grudova, R. Haines-Young, O. Inghe, T. K. Kallay, T. Kirin, H. Klug, I. P. Kokkoris, I. Konovska, M. Kruse, I. Kuzmova, M. Lange, I. Liekens, A. Lotan, D. Lowicki, S. Luque, C. Marta-Pedroso, A. Mizgajski, L. Mononen, S. Mulder, F. Müller, S. Nedkov, M. Nikolova, H. Östergård, L. Penev, P. Pereira, K. Pitkänen, T. Plieninger, S. E. Rabe, S. Reichel, P. K. Roche, G. Rusch, A. Ruskule, A. Sapundzhieva, K. Sepp, I. M. Sieber, M. Šmid Hribar, S. Stašová, B. Steinhoff-Knopp, M. Stępniewska, A. Teller, D. Vackar, M. Van Weelden, K. Veidemane, H. Vejre, P. Vihervaara, A. Viinikka, M. Villoslada, B. Weibel, and G. Zulian. 2018. Mapping and assessing ecosystem services in the EU - Lessons learned from the ESMERALDA approach of integration. One Ecosystem 3:e29153.

Cantwell, K. 2015. Okeanos Explorer ROV Dive Summary EX1806 Dive 14.

Cartapanis, O., D. Bianchi, S. L. Jaccard, and E. D. Galbraith. 2016. Global pulses of organic carbon burial in deep-sea sediments during glacial maxima. Nature Communications 7.

Cartes, J. E. 1993. Diets of deep-water pandalid shrimps on the western Mediterranean slope. Marine Ecology Progress Series 96:49–61.

Chauvet, P., A. Metaxas, and M. Matabos. 2019. Interannual variation in the population dynamics of juveniles of the deep-sea crab Chionoecetes tanneri. Frontiers in Marine Science 6:1–15.

Cheung, W. W. L., V. W. Y. Lam, J. L. Sarmiento, K. Kearney, R. Watson, and D. Pauly. 2009. Projecting global marine biodiversity impacts under climate change scenarios. Fish and Fisheries 10:235–251.

Chevaldonné, P., D. Jollivet, R. A. Feldman, D. Desbruyères, R. A. Lutz, and R. C. Vrijenhoek. 1998. Commensal scale-worms of the genus Branchipolynoe (Polychaeta: Polynoidae) at deep-sea hydrothermal vents and cold seeps. Cahiers de Biologie Marine 39:347–350.

Chevenet, F., S. Doledec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology 31:295–309.

Clark, M. R., F. Althaus, T. A. Schlacher, A. Williams, D. A. Bowden, and A. A. Rowden. 2016. The impacts of deep-sea fisheries on benthic communities: A review. ICES Journal of Marine Science 73:i51–i69.

Clayton, L., and G. Dennison. 2017. Inexpensive video drop-camera for surveying sensitive benthic habitats: Applications from glass sponge (Hexactinellida) reefs in Howe Sound, British Columbia. Canadian Field-Naturalist 131:46–54.

Cooper, L., M. Guarinello, J. Gregmeier, A. Bayard, J. Lovvorn, C. North, and J. Kolts. 2019. A video seafloor survey of epibenthic communities in the Pacific Arctic including Distributed Biological Observatory stations in the northern Bering and Chukchi seas. Deep Sea Research Part II: Topical Studies in Oceanography 162:164–179.

Corinaldesi, C. 2015. New perspectives in benthic deep-sea microbial ecology. Frontiers in Marine Science 2:1–12.

Demopoulos, A. W. J., J. R. Bourque, A. Durkin, and E. E. Cordes. 2018. The influence of seep habitats on sediment macrofaunal biodiversity and functional traits. Deep-Sea Research Part I: Oceanographic Research Papers 142:77–93.

Deutsch, C., A. Ferrel, B. Seibel, H. Portner, and R. Huey. 2015. Climate change tightens a metabolic constraint on marine habitats. Science 348:1132–1136.

Doering, P. H., C. A. Oviatt, and J. R. Kelly. 1986. The effects of the filter-feeding clam Mercenaria mercenaria on carbon cycling in experimental marine mesocosms. Journal of Marine Research 44:839–861.

Dufault, A. M., K. Marshall, and I. C. Kaplan. 2009. A synthesis of diets and trophic overlap of marine species in the California current. Page NOAA Technical Memorandum NMFS.

Dumke, I., A. Purser, Y. Marcon, S. M. Nornes, G. Johnsen, M. Ludvigsen, and F. Søreide. 2018. Underwater hyperspectral imaging as an in situ taxonomic tool for deep-sea megafauna. Scientific Reports 8:12860.

Dunlop, K. M., L. A. Kuhnz, H. A. Ruhl, C. L. Huffard, D. W. Caress, R. G. Henthorn, B. W. Hobson, P. McGill, and K. L. Smith. 2015. An evaluation of deep-sea benthic megafauna length measurements obtained with laser and stereo camera methods. Deep-Sea Research Part I: Oceanographic Research Papers 96:38–48.

Elrod, V. A., W. M. Berelson, K. H. Coale, and K. S. Johnson. 2004. The flux of iron from continental shelf sediments: A missing source for global budgets. Geophysical Research Letters 31:2–5.

Flock, M., and T. Hopkins. 1992. Species Composition, Vertical Distribution, and Food Habits of the Sergestid Shrimp Assemblage in the Eastern Gulf of Mexico. Journal of Crustacean Biology 12:210–233.

Fujita, T., and S. Ohta. 1988. Photographic observations of the life style of a deep-sea ophiuroid Asteronyx loveni (Echinodermata). Deep Sea Research Part A, Oceanographic Research Papers 35:2029–2043.

Gallo, N. D., James Cameron, Kevin Hardy, Patricia Fryer, H. B. Douglas, and A. L. Lisa. 2015. Submersible- and lander-observed community patterns in the Mariana and New Britain

trenches: Influence of productivity and depth on epibenthic and scavenging communities. Deep-Sea Research Part I: Oceanographic Research Papers 99:119–133.

Gallo, N. D., and L. A. Levin. 2016. Fish Ecology and Evolution in the World's Oxygen Minimum Zones and Implications of Ocean Deoxygenation. Page Advances in Marine Biology. First edition. Elsevier Ltd.

Gates, A. R., M. C. Benfield, D. J. Booth, A. M. Fowler, D. Skropeta, and D. O. B. Jones. 2017. Deep-sea observations at hydrocarbon drilling locations: Contributions from the SERPENT Project after 120 field visits. Deep-Sea Research Part II: Topical Studies in Oceanography 137:463–479.

Gieskes, J., A. E. Rathburn, J. B. Martin, M. E. Pérez, C. Mahn, J. M. Bernhard, and S. Day. 2011. Cold seeps in Monterey Bay, California: Geochemistry of pore waters and relationship to benthic foraminiferal calcite. Applied Geochemistry 26:738–746.

Gilly, W. F., J. M. Beman, S. Y. Litvin, and B. H. Robison. 2013. Oceanographic and Biological Effects of Shoaling of the Oxygen Minimum Zone. Annual Review of Marine Science 5:393–420.

Gogina, M., M. L. Zettler, J. Vanaverbeke, J. Dannheim, G. Van Hoey, N. Desroy, A. Wrede, H. Reiss, S. Degraer, V. Van Lancker, A. Foveau, U. Braeckman, D. Fiorentino, J. Holstein, and S. N. R. Birchenough. 2020. Interregional comparison of benthic ecosystem functioning: Community bioturbation potential in four regions along the NE Atlantic shelf. Ecological Indicators 110.

Grupe, B. M., M. L. Krach, A. L. Pasulka, J. M. Maloney, L. A. Levin, and C. A. Frieder. 2015. Methane seep ecosystem functions and services from a recently discovered southern California seep. Marine Ecology 36:91–108.

Haines-Young, R., and M. B. Potschin-Young. 2018. Revision of the common international classification for ecosystem services (CICES V5.1): A policy brief. One Ecosystem 3:e27108.

Hattori, T., T. Okuda, Y. Narimatsu, Y. Ueda, and M. Ito. 2009. Spatiotemporal variations in nutritional status and feeding habits of immature female bighand thornyhead Sebastolobus macrochir off the Pacific coast of northern Honshu, Japan. Fisheries Science 75:611–618.

Helly, J. J., and L. A. Levin. 2004. Global distribution of naturally occurring marine hypoxia on continental margins. Deep-Sea Research Part I: Oceanographic Research Papers 51:1159–1168.

Hernández-Ávila, I., E. Guerra-Castro, C. Bracho, M. Rada, F. A. Ocaña, and D. Pech. 2018. Variation in species diversity of deep-water megafauna assemblages in the Caribbean across depth and ecoregions. PLoS ONE 13:1–18.

Hidaka, K., K. Kawaguchi, M. Murakami, and M. Takahashi. 2001. Downward transport of organic carbon by diel migratory micronekton in the western equatorial Pacific: its quantitative and qualitative importance. Deep Sea Research Part I: Oceanographic Research Papers 48:1923–1939.

Hilário, A., M. Capa, T. G. Dahlgren, K. M. Halanych, C. T. S. Little, D. J. Thornhill, C. Verna, and A. G. Glover. 2011. New perspectives on the ecology and evolution of siboglinid tubeworms. PLoS ONE 6.

Hissmann, K. 2005. In situ observations on benthic siphonophores (Physonectae: Rhodaliidae) and descriptions of three new species from Indonesia and South Africa. Systematics and Biodiversity 2:223–249.

Hoeberechts, M., D. Owens, D. J. Riddell, and A. D. Robertson. 2016. The Power of Seeing: Experiences using video as a deep-sea engagement and education tool. OCEANS 2015 - MTS/IEEE Washington:1–9.

Hou, Y. R., Y. J. Sun, Q. F. Gao, S. L. Dong, B. Wen, and H. B. Yu. 2017. Effect of the bioturbation derived from sea cucumber Apostichopus japonicus (Selenka) farming on the different occurrence forms of sedimentary inorganic carbon. Aquaculture 480:108–115.

Huang, J. M., B. J. Baker, J. T. Li, and Y. Wang. 2019. New microbial lineages capable of carbon fixation and nutrient cycling in deepsea sediments of the northern South China Sea. Applied and Environmental Microbiology 85.

Hudson, J. M., D. K. Steinberg, T. T. Sutton, J. E. Graves, and R. J. Latour. 2014. Myctophid feeding ecology and carbon transport along the northern Mid-Atlantic Ridge. Deep Sea Research Part I: Oceanographic Research Papers 93:104–116.

Jacobson, L. D., and R. D. Vetter. 1996. Bathymetric demography and niche separation of thornyhead rockfish: Sebastolobus alascanus and Sebastolobus altivelis. Canadian Journal of Fisheries and Aquatic Sciences 53:600–609.

Jahnke, R. 2010. Global Synthesis. Pages 597–615 Carbon and Nutrient Fluxes in Continental Margins.

Judd, A. G. 2003. The global importance and context of methane escape from the seabed. Geo-Marine Letters 23:147–154.

Katija, K., C. A. Choy, R. E. Sherlock, A. D. Sherman, and B. H. Robison. 2017. From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea. Science Advances 3:e1700715.

Keller, A. A., L. Ciannelli, W. W. Wakefield, V. Simon, J. A. Barth, and S. D. Pierce. 2015. Occurrence of demersal fishes in relation to near-bottom oxygen levels within the California Current large marine ecosystem. Fisheries Oceanography 24:162–176.

Klevjer, T. A., X. Irigoien, A. Røstad, E. Fraile-Nuez, V. M. Benítez-Barrios, and S. Kaartvedt. 2016. Large scale patterns in vertical distribution and behaviour of mesopelagic scattering layers. Scientific Reports 6.

Koslow, J. A., G. W. Boehlert, J. D. M. Gordon, R. L. Haedrich, P. Lorance, and N. Parin. 2000. Continental slope and deep-sea fisheries: Implications for a fragile ecosystem. ICES Journal of Marine Science 57:548–557.

Lan, Y., J. Sun, W. Zhang, T. Xu, Y. Zhang, C. Chen, D. Feng, H. Wang, J. Tao, J.-W. Qiu, and P.-Y. Qian. 2019. Host–Symbiont Interactions in Deep-Sea Chemosymbiotic Vesicomyid Clams: Insights From Transcriptome Sequencing. Frontiers in Marine Science 6.

Larson, R. J., C. E. Mills, and G. R. Harbison. 1991. Western Atlantic midwater hydrozoan and scyphozoan medusae: in situ studies using manned submersibles. Hydrobiologia 216–217:311–317.

Lauerman, L. M. L. 1998. Diet and feeding behavior of the deep-water sea star Rathbunaster californicus (fisher) in the Monterey Submarine Canyon. Bulletin of Marine Science 63:523–530.

Lavaleye, M., G. Duineveld, M. Bergman, and I. van den Beld. 2017. Long-term baited lander experiments at a cold-water coral community on Galway Mound (Belgica Mound Province, NE Atlantic). Deep-Sea Research Part II: Topical Studies in Oceanography 145:22–32.

Lavik, G., T. Stührmann, V. Brüchert, A. Van Der Plas, V. Mohrholz, P. Lam, M. Mußmann, B. M. Fuchs, R. Amann, U. Lass, and M. M. M. Kuypers. 2009. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. Nature 457:581–584.

Le, J. T., L. A. Levin, and R. T. Carson. 2017. Incorporating ecosystem services into environmental management of deep-seabed mining. Deep-Sea Research Part II 137:486–503.

Levin, L. A., D. W. James, C. M. Martin, A. E. Rathburn, L. H. Harris, and R. H. Michener. 2000. Do methane seeps support distinct macrofaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. Marine Ecology Progress Series 208:21–39.

Levin, L. A. 2005. Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology 43:1–46.

Levin, L. A., and P. K. Dayton. 2009. Ecological theory and continental margins: where shallow meets deep. Trends in Ecology and Evolution 24:606–617.

Levin, L. A., A. R. Baco, D. A. Bowden, A. Colaco, E. E. Cordes, M. R. Cunha, A. W. J. Demopoulos, J. Gobin, B. M. Grupe, J. Le, A. Metaxas, A. N. Netburn, G. W. Rouse, A. R. Thurber, V. Tunnicliffe, C. L. Van Dover, A. Vanreusel, and L. Watling. 2016a. Hydrothermal vents and methane seeps: Rethinking the sphere of influence. Frontiers in Marine Science 3.

Levin, L., P. Girguis, C. German, M. Brennan, S. Tüzün, J. Wagner, C. Smart, A. Kruger, K. Inderbitzen, J. Le, M. Martinez, C. Martinez, E. Kappel, N. Gallo, and B. Grupe. 2016b. Exploration and discovery of methane seeps and associated communities in the California Borderland. New Frontiers in Ocean Exploration: The E/V Nautilus and NOAA Ship Okeanos Explorer 2015 Field Season 29:40–43.

Lindegren, M., B. G. Holt, B. R. MacKenzie, and C. Rahbek. 2018. A global mismatch in the protection of multiple marine biodiversity components and ecosystem services. Scientific Reports 8:4099.

Little, S. H., D. Vance, J. McManus, and S. Severmann. 2016. Key role of continental margin sediments in the oceanic mass balance of Zn and Zn isotopes. Geology 44:207–210.

Macreadie, P. I., D. L. McLean, P. G. Thomson, J. C. Partridge, D. O. B. Jones, A. R. Gates, M. C. Benfield, S. P. Collin, D. J. Booth, L. L. Smith, E. Techera, D. Skropeta, T. Horton, C. Pattiaratchi, T. Bond, and A. M. Fowler. 2018. Eyes in the sea: Unlocking the mysteries of the ocean using industrial, remotely operated vehicles (ROVs). Science of the Total Environment 634:1077–1091.

Maldonado, M., R. Aguilar, R. Bannister, J. Bell, K. Conway, P. Dayton, C. Diaz, J. Gutt, M. Kelly, E. Kenchington, S. Leys, S. Pomponi, H. Rapp, K. Rutzler, O. Tendal, J. Vacelet, and C. Young. 2017. Sponge ground as key marine habitats: A synthetic review of types, structure, functional roles, and conservation concerns. Page Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots.

Maloney, J. M., B. M. Grupe, A. L. Pasulka, K. S. Dawson, D. H. Case, C. A. Frieder, L. A. Levin, and N. W. Driscoll. 2015. Transpressional segment boundaries in strike-slip fault systems offshore southern California: Implications for fluid expulsion and cold seep habitats. Geophysical Research Letters 42:4080–4088.

Marlow, J. J., J. A. Steele, W. Ziebis, A. R. Thurber, L. A. Levin, and V. J. Orphan. 2014. Carbonate-hosted methanotrophy represents an unrecognized methane sink in the deep sea. Nature Communications 5.

Martinetto, P., D. I. Montemayor, J. Alberti, C. S. B. Costa, and O. Iribarne. 2016. Crab bioturbation and herbivory may account for variability in carbon sequestration and stocks in south west atlantic salt marshes. Frontiers in Marine Science 3:1–12.

Matabos, M., M. Hoeberechts, C. Doya, J. Aguzzi, J. Nephin, T. E. Reimchen, S. Leaver, R. M. Marx, A. Branzan Albu, R. Fier, U. Fernandez-Arcaya, and S. K. Juniper. 2017. Expert, Crowd, Students or Algorithm: who holds the key to deep-sea imagery 'big data' processing? Methods in Ecology and Evolution 8:996–1004.

Mejía-Mercado, B. E., B. Mundy, and A. R. Baco. 2019. Variation in the structure of the deep-sea fish assemblages on Necker Island, Northwestern Hawaiian Islands. Deep-Sea Research Part I: Oceanographic Research Papers 152:103086.

Merrett, N. R., and L. Saldanha. 1985. Aspects of the morphology and ecology of some unusual deep-sea eels (Synaphobranchidae, Derichthyidae and Nettastomatidae) from the eastern North Atlantic. Journal of Fish Biology 27:719–747.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington DC.

Miller, R., C. Smith, D. Demaster, and W. Fornes. 2000. Feeding selectivity and rapid particle processing by deep-sea megafaunal deposit feeders: A 234Th tracer approach. Journal of Marine Research 58:653–673.

Mills, C. E. 1995. Medusae, siphonophores, and ctenophores as planktivorous predators in changing global ecosystems. ICES Journal of Marine Science 52:575–581.

Mindel, B. L., F. C. Neat, C. N. Trueman, T. J. Webb, and J. L. Blanchard. 2016. Functional, size and taxonomic diversity of fish along a depth gradient in the deep sea. PeerJ:e2387.

Muller-Karger, F. E., R. Varela, R. Thunell, R. Luerssen, C. Hu, and J. J. Walsh. 2005. The importance of continental margins in the global carbon cycle. Geophysical Research Letters 32.

Myhre, S. E., D. Pak, M. Borreggine, J. P. Kennett, C. Nicholson, T. M. Hill, and C. Deutsch. 2018. Oxygen minimum zone biotic baseline transects for paleoceanographic reconstructions in Santa Barbara Basin, CA. Deep-Sea Research Part II: Topical Studies in Oceanography 150:118–131.

Naehr, T. H., P. Eichhubl, V. J. Orphan, M. Hovland, C. K. Paull, W. Ussler, T. D. Lorenson, and H. G. Greene. 2007. Authigenic carbonate formation at hydrocarbon seeps in continental margin sediments: A comparative study. Deep-Sea Research Part II: Topical Studies in Oceanography 54:1268–1291.

Neira, C., I. King, G. Mendoza, J. Sellanes, P. De Ley, and L. A. Levin. 2013. Nematode community structure along a central Chile margin transect influenced by the oxygen minimum zone. Deep-Sea Research Part I: Oceanographic Research Papers 78:1–15.

Netburn, A. N., and J. Anthony Koslow. 2015. Dissolved oxygen as a constraint on daytime deep scattering layer depth in the southern California current ecosystem. Deep-Sea Research Part I: Oceanographic Research Papers 104:149–158.

Orphan, V. J., C. H. House, K. U. Hinrichs, K. D. McKeegan, and E. F. DeLong. 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proceedings of the National Academy of Sciences of the United States of America 99:7663–7668.

Pacific Fishery Management Council. 2019. Pacific Coast Groundfish Fishery Management Plan Appendix B Part 2.

Pasulka, A. L., S. K. Goffredi, P. L. Tavormina, K. S. Dawson, L. A. Levin, G. W. Rouse, and V. J. Orphan. 2017. Colonial tube-dwelling ciliates influence methane cycling and microbial diversity within methane seep ecosystems. Frontiers in Marine Science 3.

Pearcy, W. G., and D. Hancock. 1978. Feeding habits of dover sole, Microstomus pacificus; rex sole, Glyptocephalus zachirus; slender sole, Lyopsetta exilis; and Pacific sanddab, Citharichthys sordidus, in a region of diverse sediments and bathymetry off Oregon. Fishery Bulletin 76:641–651.

Pearson, M., and J. D. Gage. 1984. Diets of some deep-sea brittle stars in the Rockall Trough. Marine Biology 82:247–258.

Peek, A. S., R. A. Feldman, R. A. Lutz, and R. C. Vrijenhoek. 1998. Cospeciation of chemoautotrophic bacteria and deep sea clams. Proceedings of the National Academy of Sciences of the United States of America 95:9962–9966.

Purcell, J. 1977. The Diet of Large and Small Individuals of the Sea Anemone Metridium senile. Bulletin of the Southern California Academy of Sciences 76.

Qin, H., X. Li, Z. Yang, and M. Shang. 2015. When underwater imagery analysis meets deep learning: A solution at the age of big visual data. OCEANS 2015 MTS/IEEE Washington:1–5.

Raineault, N. A., A. C. Trembanis, and D. C. Miller. 2012. Mapping Benthic Habitats in Delaware Bay and the Coastal Atlantic: Acoustic Techniques Provide Greater Coverage and High Resolution in Complex, Shallow-Water Environments. Estuaries and Coasts 35:682–699.

Rees, S. E., M. C. Austen, M. J. Attrill, and L. D. Rodwell. 2012. Incorporating indirect ecosystem services into marine protected area planning and management. International Journal of Biodiversity Science, Ecosystem Services and Management 8:273–285.

Reinthaler, T., H. M. van Aken, and G. J. Herndl. 2010. Major contribution of autotrophy to microbial carbon cycling in the deep North Atlantic's interior. Deep-Sea Research Part II: Topical Studies in Oceanography 57:1572–1580.

Rex, M. A. 1981. Community Structure in the Deep-Sea Benthos. Annual Review of Ecology and Systematics 12:331–353.

Riedel, M., M. Scherwath, M. Römer, M. Veloso, M. Heesemann, and G. D. Spence. 2018. Distributed natural gas venting offshore along the Cascadia margin. Nature Communications 9:3264.

Rogers, A. D. 2000. The role of the oceanic oxygen minima in generating biodiversity in the deep sea. Deep-Sea Research Part II: Topical Studies in Oceanography 47:119–148.

Rosen, D., and A. Lauermann. 2016. It's all about your network: Using ROVs to assess Marine Protected Area effectiveness. OCEANS 2016 MTS/IEEE Monterey:1–6.

Santín, A., J. Grinyó, S. Ambroso, M. J. Uriz, A. Gori, C. Dominguez-Carrió, and J. M. Gili. 2018. Sponge assemblages on the deep Mediterranean continental shelf and slope (Menorca Channel, Western Mediterranean Sea). Deep-Sea Research Part I: Oceanographic Research Papers 131:75–86.

Seabrook, S., F. C. De Leo, T. Baumberger, N. Raineault, and A. R. Thurber. 2018. Heterogeneity of methane seep biomes in the Northeast Pacific. Deep-Sea Research Part II: Topical Studies in Oceanography 150.

Seabrook, S., F. C. De Leo, and A. R. Thurber. 2019. Flipping for food: The use of a methane seep by tanner crabs (Chionoecetes tanneri). Frontiers in Marine Science 6.

Seibel, B. A. 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. Journal of Experimental Biology 214:326–336.

Sellanes, J., C. Neira, E. Quiroga, and N. Teixido. 2010. Diversity patterns along and across the Chilean margin: A continental slope encompassing oxygen gradients and methane seep benthic habitats. Marine Ecology 31:111–124.

Sibuet, M., and K. Olu. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Research Part II: Topical Studies in Oceanography 45:517–567.

Simon-Lledó, E., B. J. Bett, V. A. I. Huvenne, T. Schoening, N. M. A. Benoist, and D. O. B. Jones. 2019a. Ecology of a polymetallic nodule occurrence gradient: Implications for deep-sea mining. Limnology and Oceanography 64:1883–1894.

Smith, K. L., C. L. Huffard, P. R. McGill, A. D. Sherman, T. P. Connolly, S. Von Thun, and L. A. Kuhnz. 2019. Gelatinous zooplankton abundance and benthic boundary layer currents in the abyssal Northeast Pacific: A 3-yr time series study. Deep-Sea Research Part II: Topical Studies in Oceanography:104654.

Sperling, E. A., C. A. Frieder, and L. A. Levin. 2016. Biodiversity response to natural gradients of multiple stressors on continental margins. Proceedings of the Royal Society B: Biological Sciences 283.

Stramma, L., S. Schmidtko, L. A. Levin, and G. C. Johnson. 2010. Ocean oxygen minima expansions and their biological impacts. Deep-Sea Research Part I: Oceanographic Research Papers 57:587–595.

Stramma, L., and S. Schmidtko. 2019. Global evidence of ocean deoxygenation. Page in D. Laffoley and J. Baxter, editors. Ocean deoxygenation: Everyone's problem. IUCN.

Sweetman, A. K., C. R. Smith, C. N. Shulse, B. Maillot, M. Lindh, M. J. Church, K. S. Meyer, D. van Oevelen, T. Stratmann, and A. J. Gooday. 2019. Key role of bacteria in the short-term cycling of carbon at the abyssal seafloor in a low particulate organic carbon flux region of the eastern Pacific Ocean. Limnology and Oceanography 64:694–713.

Thurber, A. R., A. K. Sweetman, B. E. Narayanaswamy, D. O. B. Jones, J. Ingels, and R. L. Hansman. 2014. Ecosystem function and services provided by the deep sea. Biogeosciences 11:3941–3963.

Tobler, M., C. N. Passow, R. Greenway, J. L. Kelley, and J. H. Shaw. 2016. The Evolutionary Ecology of Animals Inhabiting Hydrogen Sulfide–Rich Environments. Annual Review of Ecology, Evolution, and Systematics 47:239–262.

Tobler, M., I. Schlupp, F. J. García de León, M. Glaubrecht, and M. Plath. 2007. Extreme habitats as refuge from parasite infections? Evidence from an extremophile fish. Acta Oecologica 31:270–275.

Treude, T., S. Kiel, P. Linke, J. Peckmann, and J. L. Goedert. 2011. Elasmobranch egg capsules associated with modern and ancient cold seeps: A nursery for marine deep-water predators. Marine Ecology Progress Series 437:175–181.

Vacelet, J., and E. Duport. 2004. Prey capture and digestion in the carnivorous sponge Asbestopluma hypogea (Porifera: Demospongiae). Zoomorphology 123:179–190.

Vardaro, M. F., H. A. Ruhl, and K. L. Smith. 2009. Climate variation, carbon flux, and bioturbation in the abyssal North Pacific. Limnology and Oceanography 54:2081–2088.

Werner, S. R., J. P. G. Spurgeon, G. H. Isaksen, J. P. Smith, N. K. Springer, D. A. Gettleson, L. N'Guessan, and J. M. Dupont. 2014. Rapid prioritization of marine ecosystem services and ecosystem indicators. Marine Policy 50:178–189.

Wilmers, C. C., J. A. Estes, M. Edwards, K. L. Laidre, and B. Konar. 2012. Do trophic cascades affect the storage and flux of atmospheric carbon? An analysis of sea otters and kelp forests. Frontiers in Ecology and the Environment 10:409–415.

Wishner, K. F., B. A. Seibel, C. Roman, C. Deutsch, D. Outram, C. T. Shaw, M. A. Birk, K. A. S. Mislan, T. J. Adams, D. Moore, and S. Riley. 2018. Ocean deoxygenation and zooplankton: Very small oxygen differences matter. Science Advances 4:eaau5180.

Wyrtki, K. 1962. The oxygen minima in relation to ocean circulation. Deep-Sea Research and Oceanographic Abstracts 9:11–23.

Yahel, G., R. Yahel, T. Katz, B. Lazar, B. Herut, and V. Tunnicliffe. 2008. Fish activity: A major mechanism for sediment resuspension and organic matter remineralization in coastal marine sediments. Marine Ecology Progress Series 372:195–209.

Yang, T., and G. N. Somero. 1993. Effects of Feeding and Food Deprivation on Oxygen Consumption, Muscle Protein Concentration and Activities of Energy Metabolism Enzymes in Muscle and Brain of Shallow-Living (Scorpaena Guttata) and Deep-Living (Sebastolobus Alascanus) Scorpaenid Fishes. Journal of Experimental Biology 181:213–

Chapter 5: Ecosystem services associated with natural stormwater treatment systems in Los Angeles: A review

Jennifer T. Le, Jennifer Gonzalez, Richard T. Carson, Richard Ambrose, Lisa A. Levin

Abstract

Natural stormwater treatment systems (NTS) are built ecosystems designed to capture and treat stormwater runoff via natural processes. The biological communities associated with NTS, including plants, animals, and microbes, provide non-targeted functions that can result in additional ecosystem services, such as biodiversity, pollination, and climate regulation. Additional benefits of NTS include recreation, education and outreach opportunities, and aesthetic value. A review of ecosystem services and additional benefits associated with NTS is provided with specific examples from Los Angeles County. In our discussion of how these co-benefits are provided, we highlight needs in environmental assessment and monitoring programs: indicators that can quantify ecosystem services, and standardization of measurements for comparison across space and time. As NTS become widespread in places like Los Angeles County, the ability to assess how well NTS are performing is imperative to developing best practices and optimizing benefits. We conclude by offering three models that can be used to evaluate NTS performance. Such information can be important in advancing NTS design and spatial placement, as well as in making choices between NTS and more traditional stormwater treatment options.

Introduction

Development and urbanization transform coastal landscapes by replacing vegetation with impermeable surfaces. Subsequent precipitation events can lead to decreased water infiltration, modified water flows, and introduction of contaminants into stormwater runoff (Goonetilleke et al., 2005; Walsh et al., 2005). As a result, flooding and property damage, increased safety and health risks, and environmental damage can occur (Wheater & Evans, 2009; Poff & Zimmerman, 2010; Grimm, 2011). Urban planners and developers have traditionally addressed stormwater runoff issues by building drainage systems that connect directly to large bodies of water (e.g. streams, ocean) or treatment plants. The former have been found to further alter hydrology and degrade water quality (Walsh et al., 2012), while the latter can be energetically and chemically intensive (Panepinto et al., 2016).

Natural stormwater treatment systems (NTS) are emerging as an alternative stormwater management strategy (Ambrose & Winfrey, 2015). NTS (also referred to as low-impact development or best management practices) are human-made systems that use natural processes (e.g. gravity-driven hydrology, plant absorption of water and nutrients) to capture and treat stormwater runoff. They come in different forms: bioretention systems (biofilters, bioswales, rain gardens), infiltration basins and trenches, permeable pavements, dry wells and ponds, treatment wetlands, and combinations. Table 5.1 provides a summary, although there are differing opinions on what constitutes an NTS (e.g. permeable pavements). Additionally, some of the listed systems can also treat wastewater, functioning in similar ways but obtaining water from different sources. This paper focuses primarily on bioretention systems (i.e. biofilters) because they host biological communities as part of their design.

Because NTS function as built ecosystems, they can support diverse ecosystem services, defined as direct and indirect benefits humans obtain from ecosystems (Millennium Ecosystem

Assessment, 2005; de Groot et al., 2010; Haines-Young & Potschin, 2018). Ecosystem services associated with NTS have long been acknowledged (Bolund & Hunhammar, 1999; U.S. EPA, 2012a, 2012b), but they have largely been ignored by monitoring programs and economic valuation efforts, which have been limited to targeted water functions. Co-benefits have been described for other green spaces (e.g. parks, green roofs, urban forests) such as offsetting carbon emissions (e.g. McPherson, 1992; Nowak et al., 2013), cooling local temperatures (e.g. Akbari et al., 2001; Oliveira et al., 2011), cultural services (e.g. recreation, education and outreach, aesthetic value; Hammer et al., 1974; Correll et al., 1978), and benefits to human health and well-being (Tzoulas et al., 2007; Houlden et al., 2018). The incorporation of ecosystem services and other co-benefits into environmental decision-making can present urban planners and developers with additional benefits, costs, and tradeoffs to consider in order to make optimized decisions (BenDor et al., 2018; Diaz et al., 2018).

We use Los Angeles County as a case study because it experiences periodic water crises, hosts a dense human population near the coast, and has policy in place to encourage the use of NTS. There are spatial and temporal mismatches in the supply and demand for water in California, most precipitation occurring remotely from agricultural and metropolitan hubs that need consistent sources of water irrigated and imported (Chung et al., 2002). Additionally, California droughts and water shortages are predicted to increase in frequency and magnitude due to anthropogenic climate change (Diffenbaugh et al., 2015). As a state, California has passed several propositions to protect water supply and quality (e.g. AB-1471, 2014). In 2004, Los Angeles passed Proposition O which allowed the city to issue up to $500 million to fund projects that increase local water quality (City of Los Angeles, 2018). In 2012, Los Angeles adopted its Low Impact Development Ordinance (RA-2012-0175) requiring development and

redevelopment projects that alter impervious area to mitigate runoff by capturing precipitation and utilizing natural resources where possible. As a result of these environmental conditions and political momentum, green infrastructure and NTS have been broadly distributed throughout Los Angeles County and continue to be implemented. For a list of NTS in Los Angeles County please refer to the following report: https://dornsife.usc.edu/assets/sites/291/docs/Publications/Levin_et_al._2017_Biofilter_Report.pdf. This study uses a small subset to illustrate potential ecosystem services (Figure 5.1).

Here, we review ecosystem services and co-benefits associated with NTS, including their necessary structures and functions. We use specific examples from Los Angeles County to illustrate how ecological processes found in human-made NTS can be important to human well-being. The lack of quantitative data for NTS highlights the need for robust assessment and monitoring, and we propose ecosystem services indicators as a starting point. Implementation and management of NTS can benefit greatly from ecosystem service quantification and valuation, identifying alternative design options and areas of improvement.

Targeted water services

Stormwater infiltration

NTS are designed to capture stormwater runoff for infiltration or reuse (U.S. EPA, 2000), and have been shown to be effective (Davis, 2008; Li et al., 2009). Most systems are oriented vertically, using gravity to direct water flow through several layers that generally consist of a ponding area with vegetation, porous filter media, and a drainage zone (Figure 5.2). Infiltration rates of a system can vary widely depending on variables such as size (Gilroy & McCuen, 2009; Berretta et al., 2018), age (Brown & Hunt, 2012; Le Coustumer et al., 2012), filter media (Hsieh

& Davis, 2005; Sileshi et al., 2019), and other design factors (Askarizadeh et al., 2015). Vegetation also plays a role by intercepting precipitation and water flows (Berland et al., 2017), and preventing clogging of filter media to maintain infiltration capacity (Archer et al., 2002; Hatt et al., 2009, Payne et al., 2018). Additionally, evapotranspiration by plants can account for 15-20% of inflow (Sharkey, 2006).

Urban areas with low permeable surface area can be prone to flooding and changes in groundwater recharge (Konrad, 2003). Los Angeles County lacks infrastructure to handle large volumes of stormwater (Sheng & Wilson, 2009), and flooding can occur when unexpectedly high precipitation occurs. This issue occurs in a state that experiences episodic drought (Seager et al., 2013), highlighting the need for proper stormwater management and use. Targeted stormwater infiltration by NTS can provide ecosystem services that help address these challenges.

Flood control

Flood risk is measured as a combination of hazard (i.e. runoff volume, base and peak flows, flood plain) and vulnerability (i.e. infrastructure, population density). By altering landscapes and hydrology, urbanization can lead to increased flood risk caused by heavy precipitation and storm surge (Tingsanchali, 2012). NTS have been shown to significantly reduce runoff volume and magnitude of high-flow events by capturing and storing stormwater runoff. Hatt et al. (2009) found reductions in runoff volume of 33% on average, as well as peak flow reductions of at least 80% in three bioretention systems in Australia. In another field study, Davis (2008) reported mean peak flow reductions of 49% and 58% in two test bioretention

systems, as well as delays in flow peaks which can provide urban managers with time to put mitigation measures in place.

Most Los Angeles NTS showed visual indicators of flood control services: permeable surfaces that allow for water infiltration, graded landscaping to help direct runoff, and ponding areas designed to temporarily store water. For example, Elmer Avenue Green Street was constructed specifically to address street flooding during precipitation events by incorporating bioswales, biofilters, permeable pavements, and rain barrels (Belden et al., 2012). However, additional information is needed in order to quantify these services. Measurements for flood hazard may be relevant (e.g. soil porosity, pervious surfaces, changes to magnitude and timing of flood peaks) (Brody et al., 2012), as well as those for flood vulnerability (e.g. population and area exposed to changes in flood frequency) (Arnell & Gosling, 2016). Spatial models have also been developed, mapping urban surfaces to estimate infiltration capacity (Farrugia et al., 2013).

The value of flood control has been extensively studied in the context of wetlands (e.g. Costanza et al., 2008; Barbier et al., 2013), generally by assessing differences in property damage along a spectrum of wetland area (e.g. reduction in damages as proxied by replacement cost). Brander et al. (2006) conducted a meta-analysis to estimate the value of flood control by wetlands which resulted in a median of $20-30 1995 USD per hectare annually. Watson et al. (2016) estimated a net present value of less than $100 USD per hectare of wetland annually. NTS usually operate at smaller spatial scales than major wetland restoration projects so the main unknown here is whether NTS of different designs provide more localized flood control.

Groundwater recharge

Groundwater is used by more than half of the population in the U.S. and its recharge is an essential component of the water cycle (Maupin et al., 2014). Major sources of recharge in urban environments include runoff infiltration, and leakages from the water supply and sewage systems (Vázquez-Suñé et al., 2010). Urbanization can decrease groundwater recharge due to the installation of impermeable surfaces (U.S. E.P.A., 2000; Ahiablame et al., 2012). However, the importation of large volumes of water to meet demand in highly-populated urban areas also leads to significant leakages and recharge (Lerner, 2002). These changes can also degrade water quality, e.g. leakage from sewage systems, saltwater intrusion due to lowering of the water table (Bartolino & Cunningham, 2003).

NTS can contribute to groundwater recharge by providing permeable surfaces and pore space in their filter media that allow stormwater runoff to pass into the soil subsurface. The Avalon Green Alley project in south Los Angeles is designed to allow water to percolate into the soil and recharge the water table (Lindt et al., 2014). Beneath the permeable pavement of the green alley, catch basins store stormwater temporarily where it can be bioremediated before flowing into surrounding soil. Quantifying groundwater recharge can be challenging, but several methods exist to do so. Scanlon et al. (2002) describe three categories of methods: physical techniques such as measuring flows (e.g. Kazmierczak et al., 2016), tracer techniques which include the use of chemical, isotopic, or historical tracers (Vázquez-Suñé et al., 2010; Cartwright et al., 2017), and numerical modeling techniques (e.g. Reitz et al., 2017). Challenges still exist such as those posed by spatial and temporal variability (Simmers, 1997), but there are efforts to address them. For example, Tubau et al. (2017) developed a temporally explicit model for quantifying groundwater recharge.

The value of groundwater recharge can be calculated using various methods; the most appropriate is dependent on the end-use of water. For example, in 2010, California withdrew 12,700 million gallons of groundwater per day of which approximately 22.2% was used for domestic purposes (Maupin et al., 2014). The value of groundwater recharge can therefore be associated with the price of water to consumers. Replacement costs can also be used to assign a value to groundwater recharge. Artificial groundwater recharge is the spread of water on land to increase infiltration or the injection of water directly into the aquifer (Todd, 1959). These actions have associated costs and can be used to estimate a value for the same service performed by NTS.

Improved water quality

As stormwater runoff flows over urban surfaces, it can acquire contaminants such as suspended solids, heavy metals, nutrients, and pathogens (Lee & Bang, 2000). Because southern California receives little precipitation, it allows for the build-up of contaminants and results in the "first flush" phenomenon: elevated levels of stormwater pollution at the beginning of storm events (Deletic, 1998; Lee et al., 2005). These contaminants can then enter larger bodies of water (e.g. rivers, oceans) and degrade the local environment (Ahn et al., 2005). Many highly urban areas, such as Los Angeles County, are coastal which can exacerbate this issue of water quality because stormwater runoff can drain directly into the ocean with little opportunity (i.e. distance) for treatment.

There is a range of common stormwater runoff pollutants. Industrial processes (e.g. coal combustion and metal corrosion) and traffic produce copper, lead, and zinc (Makepeace et al., 1995; Joshi & Balasubramanian, 2010). Heavy metals can persist in the environment and

accumulate in sediment, plants, and animals, leading to the degradation of environmental and human health (Appendix 5A). Major contributors to nutrient pollution (mainly nitrogen and phosphorous) are fertilizers, sewage, and erosion (Carpenter et al., 1998; Yang & Toor, 2017). The input of excess nutrients into streams, lakes, and the ocean (i.e. eutrophication) can cause degradation of habitat and changes in community structure. Eutrophication can lead to harmful algal blooms, hypoxia, and anoxia, which can have cascading effects on the local ecosystem (Hallett et al., 2016). Harmful organics from herbicides, pharmaceuticals, and industrial processes (e.g. polycyclic aromatic hydrocarbons) can be found in urban stormwater runoff and be detrimental to biology (Burant et al., 2018). Pathogens also pose a risk to human health through exposure (e.g. from recreation activities and stormwater reuse). Lim et al. (2015) found that captured stormwater can be used for toilet-flushing with acceptable risk, but it does not meet required standards for showering and food-crop irrigation. Beach closures and advisories are often the result of bacteria levels exceeding water quality standards (Abdelzaher et al., 2010; McBride et al., 2013). In addition to the threat to human health, the resulting closures have associated economic costs, e.g. less use of parking lots, restaurants, and shopping (Lew & Larson, 2005; Pendleton & Kildow, 2006).

NTS remove contaminants through several pathways. Physical filtration removes debris and suspended solids (Maniquiz-Redillas et al., 2014). While this process may lead to clogging, and subsequent deterioration of bioremediation functions, informed NTS design, such as plant selection, can help maintain infiltration capacity (Payne et al., 2018). Contaminant molecules can also be removed from runoff by adsorption (sticking of contaminant molecules onto soil grains) and assimilation (uptake of contaminants by plants; Dhokpande & Kaware, 2013; Payne et al., 2014). A significant portion of bioremediation in these systems is performed by soil microbial

communities that can be stimulated by moisture (Badin et al., 2011). NTS can prevent contaminants from traveling further to pollute local bodies of water, but they can also concentrate pollutants in plants and filter media (Al-Ameri et al., 2018). These concentrated contaminants can leach into surrounding soils, e.g. due to lower oxygen levels that increase metal solubility. As a result, some maintenance is likely required in order to prevent build up and transport of contaminants into the environment.

Contaminant removal may be the most well-studied service provided by NTS. Figure 5.3 shows examples of systems installed to remove debris and contaminants from runoff. The Grand Boulevard tree wells (Venice, Los Angeles County) consists of seven water filtration systems that capture urban runoff from 6.8 acres of residential and commercial area before the water reaches the Santa Monica Bay (http://www.lastormwater.org/green-la/proposition-o/grand-boulevard-tree-wells/). The Filterra™ system (Contech Engineered Solutions) utilized on Grand Boulevard has been shown to effectively remove suspended solids, heavy metals, and nutrients, at efficiency ratios ranging from 83-88%, 33-77%, and 9-70% respectively, by comparing pollutant concentrations between inflow and outflow (Lenth et al., 2010). Removal rates of heavy metals (copper, zinc, lead, and cadmium) by bioretention systems can be quite high (above 90%) (Lim et al., 2015). Sediment and nutrient removal by NTS have also been found to be relatively high (more than 50% in most cases) in several laboratory experiments (Bratieres et al., 2008; Zinger et al., 2013). While these laboratory experiments provide a deep understanding of how NTS may function under specified conditions (e.g. dry and wet conditions, high and low temperatures), *in situ* studies are also needed to put those laboratory experiments into context. How NTS operate over time is still an open question, both over long (e.g. months to seasons) and

short (e.g. period of a storm) time scales, as well as how the timing of water quality measurements can affect results (e.g. beginning or end of the storm, post-saturation).

There are many studies that estimate the value of improved water quality using a variety of methods. Contingent valuation, including discrete choice experiments, asks a sample of respondents about their willingness-to-pay (WTP) for a spectrum of water quality (e.g. Carson & Mitchell, 1993; Chatterjee et al., 2017). A production function approach can value improved water quality by comparing the cost of alternative methods for contaminant removal, such as a stormwater treatment plant. The appropriate method is dependent on the fate of the stormwater and the final ecosystem service it provides, e.g. clean water for drinking or clean water that hosts higher biodiversity.

Non-targeted ecosystem services

There are efforts to better quantify and value the water services discussed above (e.g. Grizzetti et al., 2016), but NTS can also provide a range of non-targeted ecosystem services, linked to their utilization of natural structures and functions, and other co-benefits. Filter media and plant communities, which increase infiltration and remove contaminants, host biodiversity which contributes to ecological processes that can result in beneficial ecosystem services (Mehring & Levin, 2015). These ecosystem services and other co-benefits (or costs, in the case of disservices) are not generally considered during the design and assessment of NTS. One unique feature of NTS is that they are human-made, so they can be designed to provide specific benefits, which are discussed here to further expand design options that can enhance variety and value of services that can be provided by NTS.

Biodiversity

Vegetated NTS act as man-made ecosystems that contain a diversity of plants, animals, and microbes from which ecosystem services can be generated (Engelhardt & Ritchie, 2001; Balvanera et al., 2006; Cardinale et al., 2012; Science for Environment Policy, 2015) (Figure 5.4). They provide patches of habitat within an urban landscape, and potentially act as corridors through which organisms can move. This can be important for population connectivity and resilience in a changing environment due to removal of natural habitat, habitat fragmentation, and anthropogenic climate change (Aavik & Helm, 2018). Biodiversity is here loosely defined to encompass plant, animal, and microbial species richness, abundance, and distribution.

While NTS biodiversity can be measured using a range of methods (e.g. visual surveys, fauna collections), how biodiversity translates into ecosystem services is more complex and will need targeted studies. Plants act as ecosystem engineers in bioretention systems, influencing both hydrological and ecological features. Vegetation captures precipitation (Berland et al., 2017), undergoes evapotranspiration (Katul et al., 2012), maintains media porosity with roots, and assimilates pollutants (Payne et al., 2018). Plants determine photosynthesis and respiration rates, organic matter in soil, and ultimately, carbon sequestration and storage in NTS (Heimann & Reichstein, 2008). Additionally, plant communities influence microbial and infaunal communities which subsequently impact ecosystem function. Microbial communities are most often assessed in terms of harmful taxa present (e.g. fecal-indicator bacteria; Paule-Mercado et al., 2016) or as functional groups (i.e. denitrification, greenhouse gas emissions; McPhillips & Walter, 2015). Fauna, except in wetland settings, are usually not considered in NTS design, despite their role in facilitating targeted functions and services. Kazemi et al. (2011) and Mehring et al. (2016) identify common biofilter taxa, such as Megadrilacea (earthworms),

Enchytraeidae (potworms), and Collembola (springtails). Earthworms are known to increase water infiltration via burrows (Shipitalo & Bayon, 2004), while springtails can impact plant growth and nutrient cycling (Mehring & Levin, 2015). As a result, these soil invertebrates can be considered ecosystem engineers that move and aerate soil, shaping the microbial, floral, and faunal communities from which more ecosystem services can stem. Because urban NTS can receive more water than native ecosystems, they can host elevated biodiversity of local soil invertebrates relative to natural habitats (Ge et al., 2019). Higher biodiversity can be beneficial if they enhance service provision, or detrimental if they involve invasive species that disrupt function. The Ballona Freshwater Marsh is reported to have provided breeding and foraging grounds for 217 bird species in 2012 (Read & Strecker, 2013). Wetland bird species are used internationally to designate areas for conservation (Ramsar, 2016) and, in addition to contributing to wetland biodiversity, provide recreational services (Jenkins et al., 2010). Increases in biodiversity have also been shown to increase human well-being in urban settings (Carrus et al., 2015).

Studies exist on the economic value of biodiversity in urban and engineered settings (e.g. constructed wetlands, agriculture). These often employ contingent valuation, travel cost analysis, and hedonic property pricing methods to estimate society's WTP for biodiversity and its conservation. Travel cost analysis equates the value of an amenity with the amount of resources (e.g. money, time, opportunity cost) used to enjoy that amenity. Hedonic pricing considers goods as a bundle of attributes that can then be manipulated in order to determine how people value those attributes. In urban areas, Dupras et al. (2014) estimated that urban forests in the Greater Montreal area in Canada host biodiversity that creates a value of $2623 2013 CAD per hectare annually. Brander et al. (2006) conducted a meta-analysis of wetland biodiversity that resulted in

an average value of $17,000 1995 USD per hectare of wetland annually. Biodiversity associated with other human-made ecosystems, such as agricultural land, may also be relevant to constructed NTS (e.g. Thrupp, 2000; Pascual & Perrings, 2007; Gallai et al., 2009).

Climate regulation related to carbon

Carbon dioxide emissions are the largest contributor to anthropogenic climate change (IPCC, 2014), and as a result, climate-regulating services related to carbon (i.e. carbon sequestration and storage), have become increasingly important especially in urban areas that contribute disproportionately to global emissions (Global Platform for Sustainable Cities, 2019). NTS plants have the potential to contribute to this effort by converting atmospheric carbon dioxide into biomass through photosynthesis. How long this carbon is subsequently stored is dependent on several factors. While some carbon is quickly rereleased during respiration, some is stored as plant biomass and soil detritic compounds. Turnover rates vary with types of biomass. For example, woody biomass has slower turnover rates than biomass that contains chlorophyll (Galbraith et al., 2013). Turnover rates can also vary with soil moisture (Jabro et al., 2008), soil oxygenation, soil organic matter (Frouz et al., 2009), and microbial communities. Some bioretention systems contain saturated or submerged zones, designed to create anaerobic conditions for denitrification (Zhang et al., 2011; Zinger et al., 2013), but may also help prevent microbial breakdown of organic matter.

Quantifying carbon sequestration and storage would necessitate measurements that include net carbon fluxes (carbon dioxide and methane), soil and plant carbon density (Davies et al., 2011), and biomass turnover rates. This has been done in urban green spaces but not specifically in NTS. Nowak et al. (2013) estimated annual carbon sequestration in U.S. urban

forests to be 25.6 million tonnes which, at $36 2015 USD per tonne of carbon (U.S. EPA, 2016), has a value of over $900 million 2015 USD annually. On smaller scales, green roofs have also been shown to sequester 375 g per m^2 annually, additionally decreasing carbon emissions due to lowered electricity usage for cooling (Getter et al., 2009). Researchers at the University of California, San Diego, are currently working on evaluating net greenhouse gas fluxes over different urban landscapes, including NTS, that can potentially be associated with an economic value.

Micro-climate regulation

In addition to physically storing carbon, increased green space can reduce air and surface temperatures, reducing electricity use and emission of greenhouse gases (Shashua-Bar et al., 2009). The urban heat island effect occurs due to increased air temperature in urban settings, relative to undeveloped areas, as a result of replacing vegetation with pavement (Oke, 1983; Arnfield, 2003). Pavements, such as asphalt and cement, have lower surface albedo than natural vegetation and therefore absorb more heat. Evapotranspiration also contributes to plant regulation of micro-climates by increasing the amount of water in the air for a cooling effect. Additionally, vegetation can provide shade. Even small green spaces (e.g. neighborhood parks) can have a significant impact on micro-climate (Oliveira et al., 2011). Factors that affect micro-climate regulating ecosystem services include UV intensity, wind, and size of the green space.

The value of micro-climate regulation by urban green space can, in many instances, be calculated using avoided costs methods. For example, if a green space makes an area cooler, people may not run their air conditioning as long or intensively. The energy savings produced

could be used to assign an economic value to the micro-climate regulating services provided by the green space.

Pollination

Animal-mediated pollination is an important ecological process that supports many benefits (Morse & Calderone, 2003; Adler et al., 2001). For example, bee pollination can increase agricultural crop quantity, food quality, and market value (Klatt et al., 2014). However, bee populations have been declining due to pollution, higher pathogen prevalence, and lower genetic diversity (Potts et al., 2010; Cameron et al., 2011; Whitehorn et al., 2012). Additionally, development and urbanization cause habitat fragmentation which can lead to changes in species and functional diversity (Haddad et al., 2015). NTS can provide patches of habitat and refuge for animals within urban areas. Pollinators, such as bees and birds, can connect these habitat patches as they move along plants to feed and collect pollen (Figure 5.5). Increased suitable habitat and connectivity may facilitate the recovery of pollinator populations and create more resilient communities that can recover from disturbances, e.g. disease or long periods of intense drought (Kremen et al., 2007). Habitat patches have been shown to maintain distinct bee communities that, in aggregate, retain a significant amount of local species diversity (Hung et al., 2018).

The value of pollination services is typically associated with increases in agricultural productivity (e.g. Winfree et al., 2011; Giannini et al., 2015; IPBES, 2016), but has also been evaluated in urban settings. Breeze et al. (2015) estimated WTP for non-market pollination services in the United Kingdom using a discrete choice experiment. Their estimates suggest taxpayers are willing to pay 13.4£ annually per person to maintain these benefits. Visual indicators for potential pollination services include the presence of animal-pollinators (e.g. bees,

birds) as well as flowering plants. Quantification of this service requires data on the frequency of animal-to-flower visits as well as connectivity among NTS and other areas.

Other co-benefits

The following co-benefits have been separated from other ecosystem services because their provision does not require any ecological processes. However, biodiversity and ecological processes associated with NTS can enhance these co-benefits.

Recreation

NTS can have built-in, public spaces that human communities can utilize. Walking trails, bike lanes, benches, and wildlife viewing sites can provide recreational services (Figure 5.6). Urban green spaces have been linked to improvements in physical and mental health (Tzoulas et al., 2007; Lee & Maheswaran, 2011; James et al., 2016; Houlden et al., 2018). They have also been linked to more active and healthy lifestyles overall (Coombes et al., 2010; Veitch et al., 2012). In Los Angeles County, which has the third highest population density in California (U.S. Census Bureau, 2010), these types of spaces can be important in the midst of a densely-populated and highly-urban area. The travel cost method is most often used to calculate the value of recreational services, but it may not be appropriate for hyper-local neighborhood amenities because of little-to-no cost associated with access (More et al., 1988). Contingent valuation and hedonic pricing have also been employed (Brander & Koetse, 2011). The latter alludes to an equity question regarding who is paying for these spaces and who is benefitting from them. In Porto, Portugal, Graça et al. (2018) found that lower socioeconomic areas have the most green

space but they are unlikely to be developed in ways that provide services to the community. This suggests that recreational services provided by green spaces can be a luxury.

Education and outreach

NTS present an opportunity for education and public outreach regarding stormwater issues, pollution, watershed and urban ecology, urban planning and management, and climate change. For example, Ocean View Growing Grounds (OVGG) is a community garden within a food desert (defined as an area that lacks access to fresh produce and whole foods, often in low socioeconomic areas) in inland San Diego. OVGG incorporates bioswales into its landscape, meant to prevent crops from flooding. UC San Diego researchers have partnered with community leaders to host outreach events about hydrology, soil, and urban ecology. Greater understanding and awareness of NTS and the services they provide may lead to safer gardening practices and more efficient water use. In Los Angeles, many of the sites have educational information posted about the project, their goals, and their motivations (Figure 5.7). As part of the Elmer Avenue Green Street project, local residents participated in the planning and design processes, actively engaging in stormwater issues and how to address them. Scientific research on stormwater issues have proven useful, especially in developing countries where water quality is often a main concern but resources and infrastructure for large treatment plants are lacking (Barbosa et al., 2012).

Aesthetic value and other non-use values

Aesthetic value has been attributed to urban green spaces, which can increase surrounding property value (Chen et al., 2008; Wolch et al., 2014). NTS may provide similar

services to enhance visual, auditory, and olfactory environmental features. Green roofs and walls can dampen road traffic noise by up to 7.5 dBA (Van Renterghem et al., 2013). Urban green spaces provide habitat for "natural" sounds (e.g. birds) which are preferred over traffic noises (Irvine et al., 2009). Exposure to green spaces have also been associated with better cognitive development (Berman et al., 2008; Dadvand et al., 2015) and recovery from stressful life events (van den Berg et al., 2010).

Disservices and unanticipated costs

NTS can introduce undesired services, such as installation of unattractive elements, accumulation of pollutants, and proliferation of disease vectors. The aesthetics of NTS may not be favorable to all people, especially when they are not regularly maintained. Southern California receives very little precipitation which can be fatal for plants that are not drought-resistant. Additionally, plant communities in NTS by the ocean have an added stressor of salt (Puijenbroeck et al., 2017). This may highlight the need for use of native vegetation in NTS in order to facilitate their survival. Whether or not they are also the most aesthetically-pleasing is subjective. Regular maintenance can, not only help plant survival, but also prevent build-up of debris and pollutants, which NTS are designed to intercept. Debris degrades aesthetic value but other pollutants can cause environmental damage. Heavy metals can leach into surrounding soil and groundwater (Hatt et al., 2009). Ponding areas, that allow microbial communities to remove contaminants, can also provide habitat for mosquitoes which pose health hazards in the form of infectious diseases and allergies. For example, two Los Angeles NTS sites had advisories about West Nile virus and its carriers. Vegetation and soil media can also provide habitat for urban pests, such as rodents and ticks (Hansford et al., 2017).

Increases in urban green space have been linked to decreases in violent crime (Kuo & Sullivan, 2001; Bogar & Beyer, 2016), but may also provide spaces in which crime can happen, e.g. assault or vandalism. Several accounts of assault were cited in the South Los Angeles Wetland Park (http://abc7.com/news/arrest-made-in-connection-to-sex-assault-attempts-in-south-la-park/1297020/). Almost all of these issues can be avoided at some cost whether it is increased maintenance, more frequent monitoring, or a greater police presence. The main warning here is not that these costs are usually prohibitively expensive, but that they need to be considered when planning.

In summary, NTS that rely on natural structures and functions, e.g. bioretention systems and treatment wetlands, provide built ecosystems that can support a host of targeted and non-targeted benefits (Figure 5.8). While there are few data on the quantification of these benefits in NTS specifically, we can draw on examples from relevant systems to get an idea of how these processes may work and on what scale. There also exist many economic valuation techniques that can be employed to assign a value to these ecosystem services for incorporation into urban design and management. Cost is a component of NTS in which urban planners are likely interested. Total costs consist of planning and design, permitting, raw materials, installation, maintenance, and monitoring. Most NTS are implemented in order to meet water quality regulations and, although expensive, they may be cheaper than alternative approaches. Additionally, continued use of NTS may be a sign that their co-benefits can be significant. Long-term monitoring is necessary in order to assess whether the benefits discussed in here truly exist.

Potential quantitative models to assess ecosystem services in natural stormwater treatment systems

There are two distinct objectives that need to be addressed in order to assess ecosystem services associated with NTS: the first is to determine the quantity or rate at which the ecosystem service is provided (i.e. biophysical value), and the second is to determine the economic value of that quantity or rate. Targeted water services have been well-documented in laboratory settings but *in situ*, mesocosm studies are much fewer. Similarly, although non-targeted ecosystem services are acknowledged (e.g. Bolund & Hunhammar, 1999; U.S. EPA, 2012a, 2012b), quantitative field studies are only just beginning to emerge. Researchers from the University of California (Santa Barbara, Los Angeles, Riverside, Irvine, and San Diego campuses) are currently working on an interdisciplinary project that assesses the form and function of urban stormwater infrastructure in southern California, including potential ecosystem services (https://www.universityofcalifornia.edu/press-room/multicampus-research-programs-and-initiatives-awards-17m-grants-uc-faculty).

In the following section, we discuss three potential models for ecosystem services assessment in NTS: benefit transfer approaches, stochastic frontier analysis, and data envelopment analysis. Benefit transfer approaches can be used to assign physical and economic values to ecosystem services for cost-benefit analysis. The latter two approaches, represented by production frontier models, can assess how efficiently an NTS is providing ecosystem services which may be a useful assessment tool for environmental decision-makers. Although there are few data currently, these models can be used as a starting point and improved upon as more data become available.

Benefit transfer approaches

Meta-analyses provide a database on which a regression model can be built, determining the relationship between a dependent variable (e.g. the economic value of ecosystem services associated with NTS) and multiple explanatory variables. Meta-analyses are a method of synthesizing results from multiple studies on similar topics (Glass, 1976). They are often utilized when many studies exist and interpretation of their results is difficult, and so synthesis of results can be a helpful tool (e.g. Brander et al., 2007). Meta-analyses can also be useful in contrasting situations in which very few studies exist for the target system but fall within the range of systems that have been studied. For example, a keyword search of "urban carbon" (e.g. to evaluate the value of climate regulation associated with NTS) in the Environmental Valuation Reference Inventory yields 76 records but only 6 studies are specific to urban green space (Appendix 5B).

Regression models can then be built upon the information collected during meta-analyses. In the example case of ecosystem services associated with NTS, three categories of independent variables may be relevant: the biophysical and the socio-economic characteristics of the study site, and the study characteristics. Examples of potential explanatory variables include study site size, geographic location, scale of the study (e.g. local, regional, national, global), gross domestic product per capita, valuation method, and sample size.

Benefit transfer approaches use regression models to provide an estimate of the dependent variable (i.e. the value of an ecosystem service). In addition to benefit transfer based on a function estimated from a meta-analysis, a unit value or a function estimated from one study can also be used. However, the meta-analysis approach described here is preferable because it can account for differences between the policy and study sites. The "policy site" is the site of interest and "study sites" are those from which values have been transferred. The best benefit

transfer estimate will be that between policy and study sites that are identical in terms of biophysical and socio-economic characteristics. However, there are generally discrepancies among commodities, currencies, user attributes, wealth measures, and cultural differences between policy and study sites that can lead to errors (Johnston & Rosenberger, 2010). Although primary studies are ideal, they are not always feasible, given the high cost of resources and time. It is useful for government agencies to sponsor studies across NTS that provide different quantities of ecosystem services and try to value that output in a way that is likely to be more accurate for a local context.

Cost-benefit analysis

Cost-benefit analysis, a widely-used tool for decision-making, compares the economic costs and benefits of one or more decisions (Pearce et al., 2006). If the costs outweigh the benefits, then rational actors do not partake in the action, and vice-versa. However, there are additional factors that make decisions more complicated, such as the long- versus short-term, discounting, and positive and negative externalities. Cost-benefit analysis is often used in environmental decision-making but ecosystem services are often only included qualitatively because quantitative data do not exist (Daily et al., 2009). The use of benefit transfer approaches can help this process by taking a first-step towards economic valuation of ecosystem services.

Stochastic frontier analysis & data envelopment analysis

Stochastic frontier analysis (SFA) and data envelopment analysis (DEA) are economic modelling techniques that create a "best practice frontier" as a benchmark of efficiency (Charnes et al., 1978; Aigner, Lovell, & Schmidt, 1997). This benchmark is created using input and output

data from several sources (e.g. firms, projects, NTS), and creating one scenario (normalized to be the best practice frontier) against which efficiency for other scenarios can be compared and evaluated.

SFA takes the following generic form:

(Equation 5.1)

$$y_j = f(x_{1j}, x_{2j}, \ldots x_{nj}, U_j, V_j)$$

where y is the output (one ecosystem service or a bundle of weighted ecosystem services) of producer j; x_{nj} are NTS characteristics (e.g. location, size, biological community); and two independent error terms where U is technical inefficiency, a half-sided error term that accounts for deviations from the best-practice production frontier (determined by the most efficient producers), and V is a stochastic error term.

The SFA approach is a parametric economic modelling technique that allows for random shocks, or unpredicted events, within the model. The stochastic error term may make it suitable for urban systems that are subject to environmental variation, e.g. drought, precipitation events. SFA requires *a priori* assumptions regarding the production function form, i.e. the mathematical relationship between inputs and outputs (e.g. Cobb-Douglas, Leontief), and the distribution of the unobserved technical inefficiency terms, which can substantially influence results. A considerable econometric literature has been developed to help determine the adequacy of the statistical fit of a particular model. The SFA approach provides a natural measure of how inefficient a particular NTS is at providing an ecosystem service relative to other NTS configurations.

DEA is a nonparametric approach that does not allow for random shocks but it does not require prior knowledge of the production function form, which makes it useful for NTS-

associated ecosystem services. DEA combines inputs and outputs into one efficiency score that can be easily compared, but it requires an additional regression model to identify the impacts of independent variables. Both DEA and SFA are capable of handling multiple outputs but in SFA, they must be collapsed into one basket of outputs (i.e. ecosystem services) whose weights can have a significant impact on results. One way to weight outputs is by their economic values. However, economic values for most ecosystem services associated with NTS have not been assigned, i.e. why benefit transfer approaches may be helpful. The lack of standardized monitoring data may make a nonparametric DEA approach more feasible at this time, in addition to the SFA requirement to specify a production function form. DEA also better handles multiple outputs relative to SFA and undesirable outputs, such as negative externalities (i.e. disservices) (Chambers et al., 1998).

Benefit transfer approaches, SFA, and DEA yield results that can evaluate how well an NTS site is performing and how to improve their functioning, but have associated advantages and disadvantages (Table 5.2). Benefit transfer approaches can provide an estimate for ecosystem services value, which can be used in cost-benefit analyses to determine net changes to well-being. In addition, benefit transfer can identify drivers of change through the application of regression models. Regardless of net costs or benefits, there exists a maximum level of ecosystem services provision that can be provided with given inputs. SFA and DEA can help determine whether systems are performing at this maximum level and where improvements can be made. Both methods apply somewhat similar regression models to benefit transfer approaches, yielding quantitative relationships among variables. Other options include a fixed effects model to estimate the best-practice frontier (Schmidt and Sickles, 1984), and the multi-

product distance function which allows for a stochastic error term and multiple outputs including negative externalities (Morrison Paul et al., 2000).

In prioritizing ecosystem services, geographic location can dictate physical and social factors that can influence their value. For example, water issues have long been important in southern California and they are predicted to become more contentious due to climate change. Further, new policies in California put an emphasis on climate-regulating services. The region is also a biodiversity hotspot at risk of alteration (Calsbeek et al., 2003; Loarie et al., 2008). Urbanization has reduced the amount of suitable habitat for organisms as natural ecosystems are replaced by human structures. Urban green spaces such as NTS can be important patches of habitat and act as corridors for organisms seeking refuge (Christie & Knowles, 2015).

One of the biggest challenges in characterizing and valuing ecosystem services associated with NTS is the lack of accessible data. There is no standard monitoring program for NTS, which makes it difficult to compare across time and across sites, and there is no monitoring of ecosystem services associated with NTS. Monitoring could help identify effective management strategies, e.g. timing of maintenance, and improve the above quantitative models for more accurate estimates of value and efficiency. I*n situ*, mesocosm studies are also needed to evaluate NTS performance under actual environmental conditions (e.g. Payne et al., 2014). Other questions that still need to be addressed include how networks of NTS compare to single systems regarding both targeted and non-targeted ecosystem services, and how NTS operate over time.

Conclusions

NTS can provide a myriad of ecosystem services that range from targeted water services to ecological and regulating services that stem from the biological communities they host. NTS

in Los Angeles County, which are becoming more widespread, exhibit the many ecosystem services that can be considered in designing these systems. Lack of standardized monitoring data and programs makes it difficult to assess whether these systems are generating services they are built to provide as well as the potential co-benefits we have identified. As more holistic assessments and monitoring programs are developed that incorporate indicators of both targeted and non-targeted ecosystem services, management of NTS can more effectively optimize their performance and identify areas for improvement. Improved assessment of NTS is becoming increasingly important to urban planning and sustainable development as their use expands. Better understanding the co-benefits NTS provide could also garner support within local governing bodies and the public. In places like southern California, where water issues are in sharp focus in policy debates, effective management of stormwater runoff can reduce flooding and improve water supply. Consideration of non-targeted ecosystem services by NTS can provide one tool for urban planners and developers to make better decisions in the public interest.

Acknowledgements

This work was supported by the University of Southern California Sea Grant [grant numbers USC NOAA 61207781, USC NOAA 75199714]; and the University of California Office of the President [grant number UCOP MRP-17-455083]. The authors would like to thank Dale Squires for insightful comments and suggestions, Brandon Winfrey for field site access, and Katie Galloway and Laura Walsh for field assistance.

Chapter 5, in part, is in preparation for submission for publication. Le, J.T., Gonzalez, J., Carson, R.T., Ambrose, R.F. and L.A. Levin. Ecosystem services associated with natural

stormwater treatment systems in Los Angeles: A review. The dissertation author was the primary investigator and author of this material.

Tables

Table 5.1. Categories and brief descriptions of natural stormwater treatment systems, adapted from definitions found in Low Impact Development Center (2010) and Long Beach Development Services (2013).

Natural Stormwater Treatment System Category	Description
Bioretention systems	Vegetated cells that capture runoff to reduce volume and remove contaminants, can be lined or unlined
Infiltration systems	Basins or trenches designed to collect and pond excess runoff temporarily before infiltration into the soil
Permeable pavements	Pervious surfaces on top of infiltration layers or storage cells
Dry ponds & wells	Underground storage cells used to capture and infiltrate runoff
Treatment wetlands	Engineered wetlands designed to capture and treat runoff

Table 5.2. Advantages and disadvantages of benefit transfer approaches, stochastic frontier analysis, and data envelopment analysis for assessing the value of ecosystem services in natural stormwater treatment systems. Adapted from Chen et al. (2015).

	Benefit transfer approaches	**Stochastic frontier analysis**	**Data envelopment analysis**
Assumptions	Policy site is equitable to study site	*A priori* production function	Deterministic approach
Error	Due to differences in site characteristics	Incorporated as stochastic variable	Cannot be separated from inefficiency
Multiple outputs	Single ecosystem service	Weighted basket of ecosystem services	Allows for multiple ecosystem services
Relationship to independent variables	Relationship built into regression model	Relationship built into regression model	Need additional regression model
Interpretation of results	Associated value and drivers	Benchmark for efficiency	Benchmark for efficiency

Figures

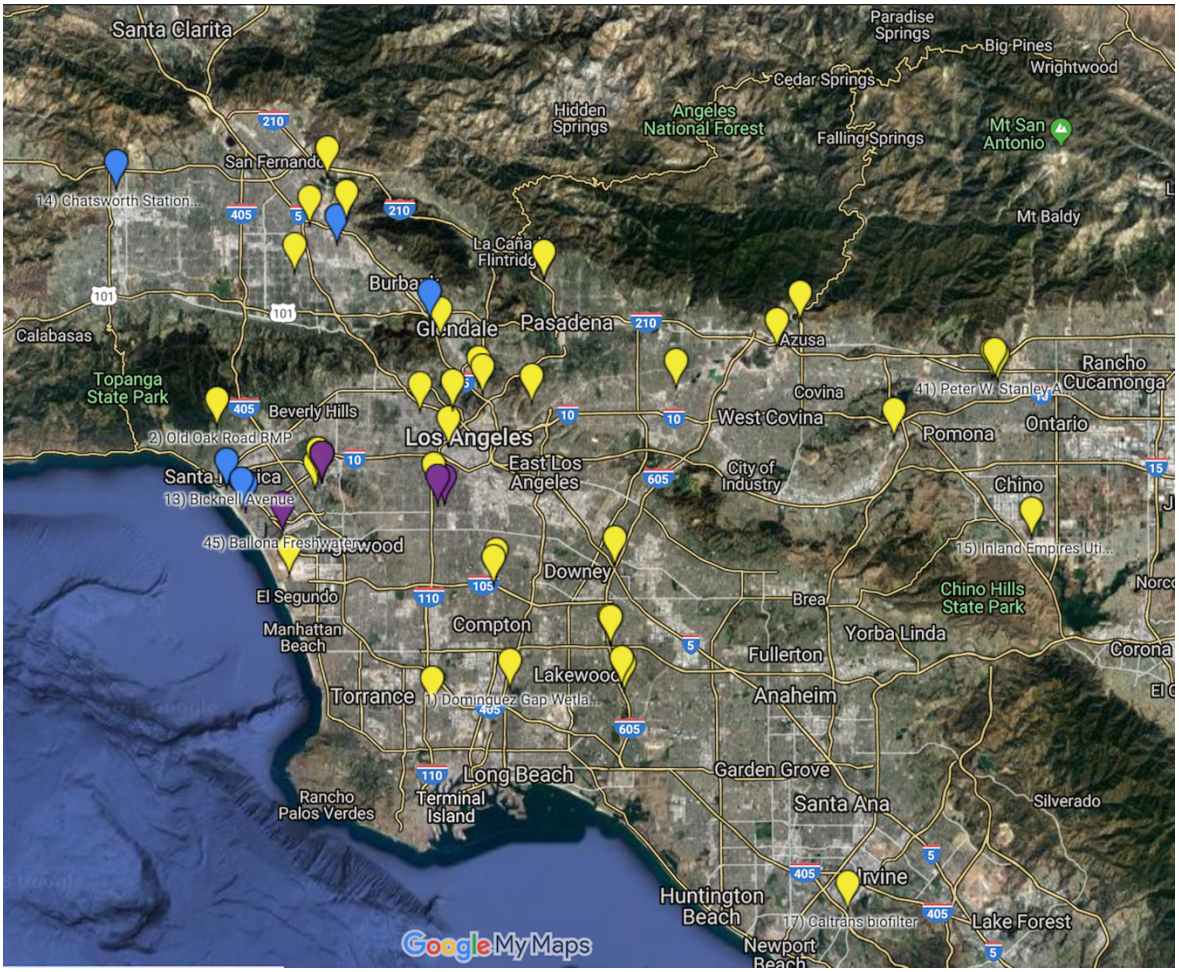

Figure 5.1. Location of natural stormwater treatment systems in Los Angeles County. Blue indicates sites visited in 2015. Purple indicates sites visited in 2016. View the interactive map here: https://goo.gl/76sswN

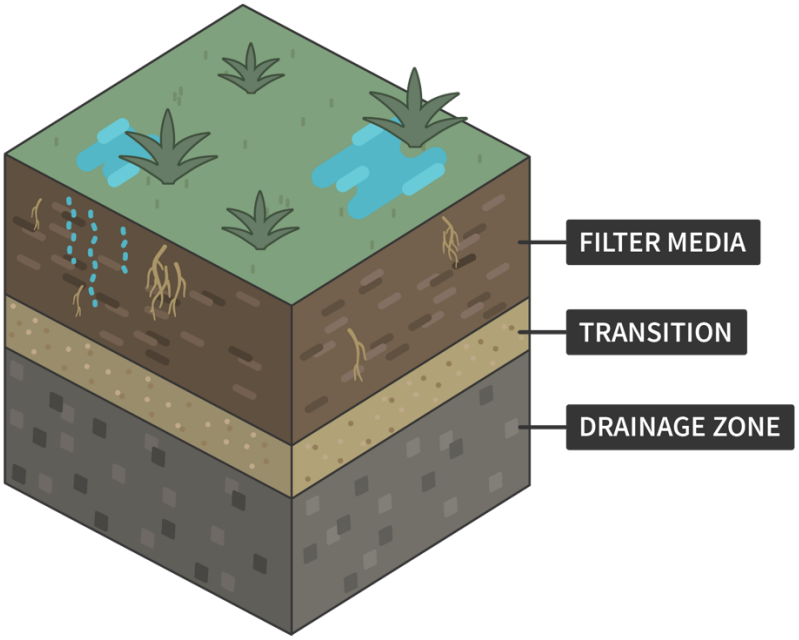

Figure 5.2. Diagram of a bioretention system with vertical layers.

Figure 5.3. Examples of Los Angeles systems designed to remove debris and contaminants from runoff: (a) StormTreat™ bioretention system at the bottom of Westminster dog park, (b) Filterra™ bioretention system on Grand Boulevard, and (c) a grate to prevent large debris from entering the stormwater system.

Figure 5.4. Examples of biodiversity that can support ecosystem services in Los Angeles natural stormwater treatment systems: (a) plant diversity at the South Los Angeles Wetland Park, (b) Bicknell manzanita tree as a bee attractant, and (c) signage illustrating the biodiversity of the Ballona Freshwater Marsh.

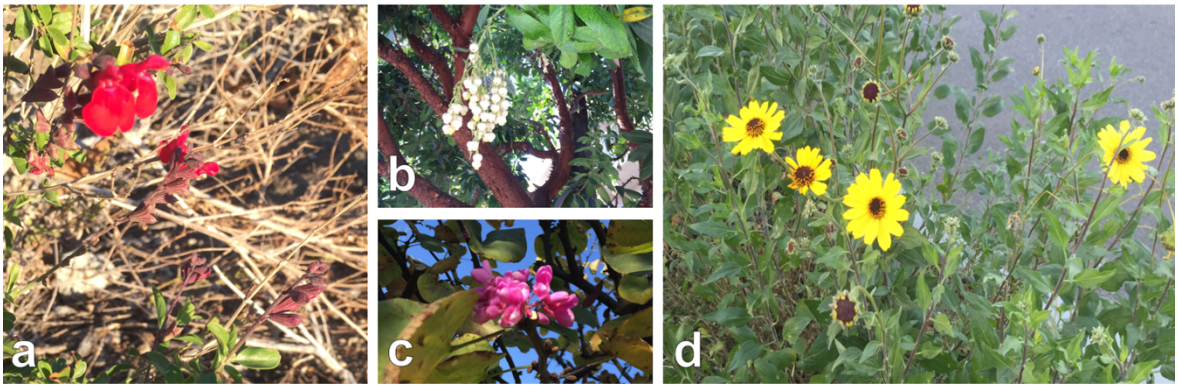

Figure 5.5. Flowering plants that could provide pollination services observed in bioretention systems at: (a) Elmer Avenue, (b) Bicknell Avenue, (c) Grand Boulevard, and (d) Los Angeles Zoo.

Figure 5.6. Visual indicators of recreation at Los Angeles natural stormwater treatment systems: (a) walking trail on Elmer Avenue Paseo, and (b-c) signage and drinking fountains at South Los Angeles Wetlands Park.

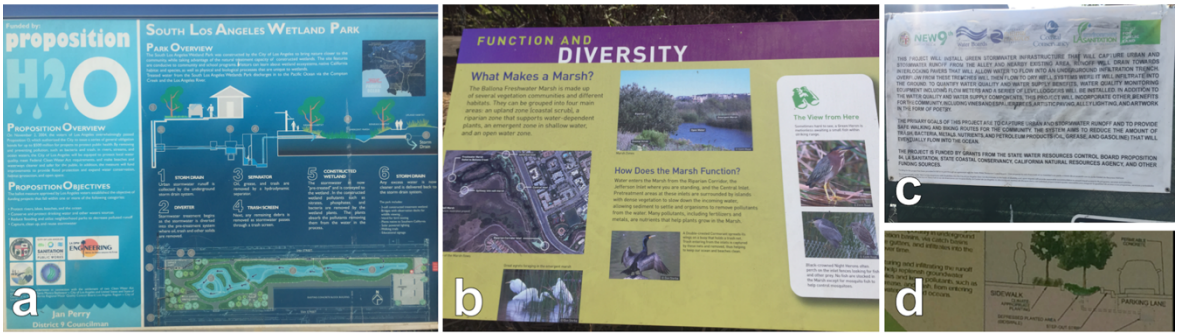

Figure 5.7. Examples of education and outreach services provided by natural stormwater treatment systems: (a) South Los Angeles Wetlands Park, (b) Ballona Freshwater Marsh, (c) Avalon Green Alley, and (d) Bicknell Avenue.

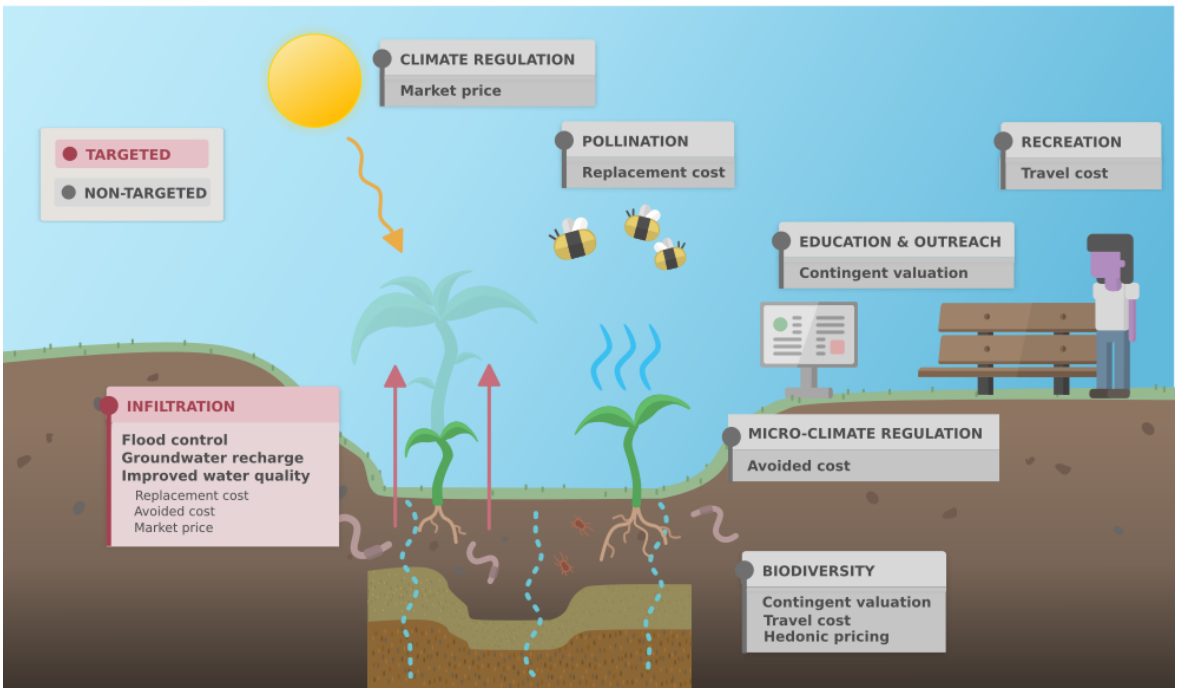

Figure 5.8. Diagram of targeted and non-targeted ecosystem services associated with natural stormwater treatment systems (a bioretention system pictured here) and methods used to assign a value to them.

Appendices

Appendix 5A. Summary of stormwater metal contaminants, their anthropogenic sources, and effects on environmental and human health.

Metal	Sources	Ecological Effects	Human Health Effects	Measured Range* [MCL]**
Aluminum	Industrial processing, coal combustion (Makepeace et al., 1995)	Toxic to animals	Nervous system, bone disease (ATSDR, 2007a); neurotoxicity (Bondy, 2010)	0.1-5540 µg/L [50 µg/L]
Arsenic	Pesticides, fungicides, preservatives, rat poison (Baby et al., 2010)	Toxic to animals, inhibition of root growth, plant sterility (Meharg & Hartley-Whitaker, 2002)	Toxic to animals, inhibition of root growth, plant sterility (Meharg & Hartley-Whitaker, 2002)	0.01-11.9 µg/L [0 µg/L]
Cadmium	Fertilizer, fossil-fuel combustion, sewage sludge, mining and industrial processing (Pan et al., 2010)	Alters plant cellular processes (Moreno et al., 1999), reduced soil microbe population	Endocrine disruptor, carcinogenic (Bernard, 2008)	0.03-2.78 µg/L [5 µg/L]
Chromium	Metal corrosion, dyes and paints, pesticides, fertilizer (Makepeace et al., 1995)	Decreases plant reproduction & growth (Shanker et al., 2005)	Carcinogenic (ASTDR, 2007b)	0.01-20 µg/L [10 µg/L] (SWRCB)
Copper	Automobiles, building corrosion (Makepeace et al., 1995)	Inhibits plant growth and development (Yruela, 2005)	Brain and kidney damage (Wuana & Okieimen, 2011)	1.2-50 µg/L (Drake et al. 2014) [1000 µg/L]
Lead	Leaded gasoline	Decreases plant reproduction (Ghani et al., 2010)	Impaired development (Wuana & Okieimen, 2011)	0.8-18 µg/L (Drake et al. 2014) [0 µg/L]
Nickel	Engines, alloy manufacturing, food production (Makepeace et al., 1995)	Inhibits plant growth and development (Chen et al., 2009)	Carcinogenic (Cempel & Nikel, 2006)	5-30.50 µg/L [100 µg/L] (SWRCB)
Zinc	Tires, metal corrosion (Makepeace et al., 1995); roofing (Brown & Peake, 2006)	Inhibits plant metabolic function (Fontes & Cox, 1998)	Dizziness, fatigue (Duruibe et al., 2007)	0.01-652 µg/L [5000 µg/L]

*Concentration ranges as reported by U.S. Geological Survey unless otherwise stated.
**Maximum contaminant loads (MCL) are goals set by the federal or state government (EPA or State Water Resources Control Board (SWRCB), respectively) derived from health-based criteria and welfare considerations. EPA MCL is reported unless otherwise stated.

Appendix 5B. Published studies and reports related to the valuation of ecosystem services described in this paper in urban and human-made systems.
https://docs.google.com/spreadsheets/d/1ASnn_8SSlE8Vga7WpaTMGfgCbVQDllqgnSvfnWmtvK0/edit?usp=sharing

References

Aavik, T., Helm, A., 2018. Restoration of plant species and genetic diversity depends on landscape-scale dispersal. Restoration Ecology, 26(S2), S92-S102. DOI: 10.1111/rec.12634

Abdelzaher, A.M., Wright, M.E., Ortega, C., Solo-Gabriele, H.M., Miller, G., Elmir, S., Newman, X., Shih, P., Alfredo Bonilla, J., Bonilla, T.D., Palmer, C.J., Scott, T., Lukasik, J., Harwood, V.J., McQuaig, S., Sinigalliano, C., Gidley, M., Plano, L.R.W., Zhu, X., Wang, J.D., Fleming, L.E., 2010. Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach. Appl. Environ. Microbiol. 76, 724–732. https://doi.org/10.1128/AEM.02127-09

Adler, L.S., Karban, R., Strauss, S.Y., 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. Ecology 82, 2032–2044.

Agency for Toxic Substances and Disease Registry, 2008. Toxicological Profile for Aluminum. Atlanta, USA.

Agency for Toxic Substances and Disease Registry, 2012. Toxicological Profile for Chromium. Atlanta, USA.

Ahiablame, L.M., Engel, B.A., Chaubey, I., 2012. Effectiveness of low impact development practices: Literature review and suggestions for future research. Water. Air. Soil Pollut. 223, 4253–4273. https://doi.org/10.1007/s11270-012-1189-2

Ahn, J.H., Grant, S.B., Surbeck, C.Q., DiGiacomo, P.M., Nezlin, N.P., Jiang, S., 2005. Coastal Water Quality Impact of Stormwater Runoff from an Urban Watershed in Southern California. Environ. Sci. Technol. 39, 5940–5953. https://doi.org/10.1021/es0501464

Aigner, D., Lovell, C.A.K., Schmidt, P., 1977. Formulation and estimation of stochastic frontier production function models. J. Econom. 6, 21–37. https://doi.org/10.1016/0304-4076(77)90052-5

Akbari, H., Pomerantz, M., Taha, H., 2001. Cool surfaces and shade trees to reduce energy use and improve air quality in urban areas. Sol. Energy 70, 295–310. https://doi.org/10.1016/S0038-092X(00)00089-X

Al-Ameri, M., Hatt, B., Le Coustumer, S., Fletcher, T., Payne, E., Deletic, A., 2018. Accumulation of heavy metals in stormwater bioretention media: A field study of temporal and spatial variation. J. Hydrol. https://doi.org/10.1016/j.jhydrol.2018.03.027

Ambrose, R.F., Winfrey, B.K., 2015. Comparison of stormwater biofiltration systems in Southeast Australia and Southern California. Wiley Interdiscip. Rev. Water 2, 131–146. https://doi.org/10.1002/wat2.1064

Archer, N.A.L., Quinton, J.N., Hess, T.M., 2002. Below-ground relationships of soil texture, roots and hydraulic conductivity in two-phase mosaic vegetation in South–east Spain. Journal of Arid Environments 52, 535–556.

Arnell, N.W., Gosling, S.N., 2016. The impacts of climate change on river flood risk at the global scale. Clim. Change 134, 387–401. https://doi.org/10.1007/s10584-014-1084-5

Arnfield, A.J., 2003. Two decades of urban climate research: A review of turbulence, exchanges of energy and water, and the urban heat island. Int. J. Climatol. 23, 1–26. https://doi.org/10.1002/joc.859

Askarizadeh, A., Rippy, M.A., Fletcher, T.D., Feldman, D.L., Peng, J., Bowler, P., Mehring, A.S., Winfrey, B.K., Vrugt, J.A., Aghakouchak, A., Jiang, S.C., Sanders, B.F., Levin, L.A., Taylor, S., Grant, S.B., 2015. From Rain Tanks to Catchments: Use of Low-Impact Development To Address Hydrologic Symptoms of the Urban Stream Syndrome. Environ. Sci. Technol. 49, 11264–11280. https://doi.org/10.1021/acs.est.5b01635

Baby, J., Raj, J.S., Biby, E.T., Sankarganesh, P., Jeevitha, M.V., Rajan, S.S., 2010. Toxic effect of heavy metals on aquatic environment. Int. J. Biol. Chem. Sci. 4, 939–952. https://doi.org/10.4314/ijbcs.v4i4.62976

Badin, A.L., Monier, A., Volatier, L., Geremia R.A., Delolme, C., Bedell J.P., 2011. Structural stability, microbial biomass and community composition of sediments affected by the hydric dynamics of an urban stormwater infiltration basin. Environmental Microbiologcy. 61:885-897. Doi: 10.1007/s00248-011-9829-4.

Balvanera, P., Pfisterer, A., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., Schmid, B., 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9, 1146–56.

Barbier, E.B., Georgiou, I.Y., Enchelmeyer, B., Reed, D.J., 2013. The Value of Wetlands in Protecting Southeast Louisiana from Hurricane Storm Surges. PLoS One 8. https://doi.org/10.1371/journal.pone.0058715

Barbosa, A.E., Fernandes, J.N., David, L.M., 2012. Key issues for sustainable urban stormwater management. Water Res. 46, 6787–6798. https://doi.org/10.1016/j.watres.2012.05.029

Bartolino, J., Cunningham, W., 2003. Ground-Water Depletion Across the Nation, U.S Geological Survey Fact Sheet 103-03. https://doi.org/10.3133/fs10303

Belden, E., Antos, M., Morris Kristy, Steele, N.L.C., 2012. Sustainable Infrastructure: The Elmer Avenue Neighborhood Retrofit, Urban Coast.

BenDor, T.K., Shandas, V., Miles, B., Belt, K., Olander, L., 2018. Ecosystem services and U.S. stormwater planning: An approach for improving urban stormwater decisions. Environ. Sci. Policy 88, 92–103. https://doi.org/10.1016/j.envsci.2018.06.006

Berland, A., Shiflett, S.A., Shuster, W.D., Garmestani, A.S., Goddard, H.C., Herrmann, D.L., Hopton, M.E., 2017. The role of trees in urban stormwater management. Landsc. Urban Plan. 162, 167–177. https://doi.org/10.1016/j.landurbplan.2017.02.017

Berman, M.G., Jonides, J., Kaplan, S., 2008. The Cognitive Benefits of Interacting with Nature. Psychological Science, 19(12), 1207-1212.

Bernard, A., 2008. Cadmium & its adverse effects on human health. Indian J. Med. Res. 128, 557–564.

Berretta, C., A, A., HS, J., Al., E., 2018. Influenc of design and media amendments on the performance of stormwater biofilters. Proc. ICE - Water Manag. 171, 87–98.

Bogar, S., Beyer, K.M., 2016. Green Space, Violence, and Crime: A Systematic Review. Trauma, Violence, Abus. 17, 160–171. https://doi.org/10.1177/1524838015576412

Bolund, P., Hunhammar, S., 1999. Ecosystem services in urban areas. Ecol. Econ. 29, 293–301.

Bondy, S.C., 2010. The neurotoxicity of environmental aluminum is still an issue. Neurotoxicology 31, 575–581. https://doi.org/10.1016/j.neuro.2010.05.009

Brander, L.M., Florax, R.J.G.M., Vermaat, J.E., 2006. The empirics of wetland valuation: A comprehensive summary and a meta-analysis of the literature. Environ. Resour. Econ. 33, 223–250. https://doi.org/10.1007/s10640-005-3104-4

Brander, L.M., Van Beukering, P., Cesar, H.S.J., 2007. The recreational value of coral reefs: A meta-analysis. Ecol. Econ. 63, 209–218. https://doi.org/10.1016/j.ecolecon.2006.11.002

Brander, L.M., Koetse, M.J., 2011. The value of urban open space: Meta-analyses of contingent valuation and hedonic pricing results. J. Environ. Manage. 92, 2763–2773. https://doi.org/10.1016/j.jenvman.2011.06.019

Bratieres, K., Fletcher, T.D., Deletic, A., Zinger, Y., 2008. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Res. 42, 3930–3940. https://doi.org/10.1016/j.watres.2008.06.009

Breeze, T.D., Bailey, A.P., Potts, S.G., Balcombe, K.G., 2015. A stated preference valuation of the non-market benefits of pollination services in the UK. Ecol. Econ. 111, 76–85. https://doi.org/10.1016/j.ecolecon.2014.12.022

Brody, S.D., Peacock, W.G., Gunn, J., 2012. Ecological indicators of flood risk along the Gulf of Mexico. Ecol. Indic. 18, 493–500. https://doi.org/10.1016/j.ecolind.2012.01.004

Brown, J.N., Peake, B.M., 2006. Sources of heavy metals and polycyclic aromatic hydrocarbons in urban stormwater runoff. Sci. Total Environ. 359, 145–155. https://doi.org/10.1016/j.scitotenv.2005.05.016

Brown, R.A., Hunt, W.F., 2012. Improving bioretention/biofiltration performance with restorative maintenance. Water Sci. Technol. 65, 361–367. https://doi.org/10.2166/wst.2012.860

Burant, A., Selbig, W., Furlong, E.T., Higgins, C.P., 2018. Trace organic contaminants in urban runoff: associations with urban land-use. Environmental Pollution. 242: 2068-2077. https://doi.org/10.1016/j.envpol.2018.06.066.

Calsbeek, R., Thompson, J.N., Richardson, J.E., 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: The California Floristic Province. Mol. Ecol. 12, 1021–1029. https://doi.org/10.1046/j.1365-294X.2003.01794.x

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., 2011. Patterns of widespread decline in North American bumble bees. Proc. Natl. Acad. Sci. 108, 662–667. https://doi.org/10.1073/pnas.1014743108

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. https://doi.org/10.1038/nature11148.Access

Carpenter, S., Caraco, N., Correll, D., Howarth, R., Sharpley, A., Smith, V., 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecol. Appl. 8, 559–568. https://doi.org/10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2

Carrus, G., Scopelliti, M., Lafortezza, R., Colangelo, G., Ferrini, F., Salbitano, F., Agrimi, M., Portoghesi, L., Semenzato, P., Sanesi, G., 2015. Go greener, feel better? The positive effects of biodiversity on the well-being of individuals visiting urban and peri-urban green areas. Landsc. Urban Plan. 134, 221–228. https://doi.org/10.1016/j.landurbplan.2014.10.022

Carson, R.T., Mitchell, R.C., 1993. The value of clean water: The public's willingness to pay for boatable, fishable, and swimmable quality water. Water Resour. Res. 29, 2445–2454.

Cartwright, I., Cendón, D., Currell, M., Meredith, K., 2017. A review of radioactive isotopes and other residence time tracers in understanding groundwater recharge: Possibilities, challenges, and limitations. J. Hydrol. 555, 797–811. https://doi.org/10.1016/j.jhydrol.2017.10.053

Cempel, M., Nikel, G., 2006. Nickel: A review of its sources and environmental toxicology. Polish J. Environ. Stud. 15, 375–382. https://doi.org/10.1109/TUFFC.2008.827

Chambers, R.G., Chung, Y., Fare, R., 1998. Profit, Directional Distance Functions, and Nerlovian Efficiency. Journal of Optimization Theory and Application. 98(2), 351-364. https://doi.org/10.1023/A:1022637501082

Charnes, A., Cooper, W., Rhodes, E., 1978. Measuring the efficiency of decision making units. Eur. J. Oper. Res. 2, 429–444. https://doi.org/10.1016/0377-2217(78)90138-8

Chatterjee, C., Triplett, R., Johnson, C.K., Ahmed, P., 2017. Willingness to pay for safe drinking water: A contingent valuation study in Jacksonville, FL. J. Environ. Manage. 203, 413–421. https://doi.org/10.1016/j.jenvman.2017.08.008

Chen, W., Jim, C.Y., 2008. Assessment and valuation of ecosystem services provided by urban forests, in: Carreiro, M., Song, Y.-C., Wu, J. (Eds.), Ecology, Planning, and Management of Urban Forests: International Perspectives. Springer Science+Business Media, LLC, New York, USA, pp. 53–83.

Chen, C., Delmas, M., Lieberman, M., 2015. Production frontier methodologies and efficiency as a performance measure in strategic management reseach. Strateg. Manag. J. 36, 19–36. https://doi.org/10.1002/smj.2199

Christie, M.R., Knowles, L.L., 2015. Habitat corridors facilitate genetic resilience irrespective of species dispersal abilities or population sizes. Evol. Appl. 8, 454–463. https://doi.org/10.1111/eva.12255

Chung, F., Kelly, K., Guivetchi, K., 2002. Averting a California Water Crisis. J. Water Resour. Plan. Manag. 128, 237–239.

City of Los Angeles, 2018. Clean Water Initiative Update. Los Angeles, USA.

Coombes, E., Jones, A.P., Hillsdon, M., 2010. The relationship of physical activity and overweight to objectively measured green space accessibility and use. Soc. Sci. Med. 70, 816–822. https://doi.org/10.1016/j.socscimed.2009.11.020

Correll, M.R., Lillydahl, J.H., Singell, L.D., 1978. The Effects of Greenbelts on Residential Property Values: Some Findings on the Political Economy of Open Space. Land Econ. 54, 207–217. https://doi.org/10.2307/3146234

Costanza, R., Pérez-Maqueo, O., Martinez, M.L., Sutton, P., Anderson, S.J., Mulder, K., 2008. The Value of Coastal Wetlands for Hurricane Protection. AMBIO A J. Hum. Environ. 37, 241–248. https://doi.org/10.1579/0044-7447(2008)37[241:TVOCWF]2.0.CO;2

Dadvand, P., Nieuwenhuijsen, M.J., Esnaola, M., Forns, J., Basagaña, X., Alvarez-Pedrerol, M., Rivas, I., López-Vicente, M., De Castro Pascual, M., Su, J., Jerrett, M., Querol, X., Sunyer, J., 2015. Green spaces and cognitive development in primary schoolchildren. Proc. Natl. Acad. Sci. 112, 7937–7942. https://doi.org/10.1073/pnas.1503402112

Daily, G.C., Polasky, S., Goldstein, J., Kareiva, P.M., Mooney, H.A., Pejchar, L., Ricketts, T.H., Salzman, J., Shallenberger, R., 2009. Ecosystem services in decision making: Time to deliver. Front. Ecol. Environ. 7, 21–28. https://doi.org/10.1890/080025

Davies, Z.G., Edmondson, J.L., Heinemeyer, A., Leake, J.R., Gaston, K.J., 2011. Mapping an urban ecosystem service: quantifying above-ground carbon storage at a city-wide scale. J. Appl. Ecol. 48, 1125–1134.

Davis, A.P., 2008. Field Performance of Bioretention : Hydrology Impacts. J. Hydrol. Eng. 13, 90–95.

de Groot, R., Fisher, B., Christie, M., Aronson, J., Braat, L., Gowdy, J., Haines-Young, R., Maltby, E., Neuville, A., Polasky, S., Portela, R., Ring, I., 2010. Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation. In: Kumar, P. (Ed.), The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. Earthscan, London and Washington.

Deletic, A., 1998. The first flush load of urban surface runoff. Water Res. 32, 2462–2470. https://doi.org/10.1016/S0043-1354(97)00470-3

Dhokpande, S.R., Kaware, J.P., 2013. Biological Methods for Heavy Metal Removal- A Review. Int. J. Eng. Sci. Innov. Technol. 2, 304–309.

Díaz, B.S., Pascual, U., Stenseke, M., Martín-lópez, B., Watson, R. T., Molnár, Z, et al., 2018. Assessing nature's contributions to people. Science. 359(6373): 270-272. doi: 10.1126/science.aap8826.

Diffenbaugh, N.S., Swain, D.L., Touma, D., 2015. Anthropogenic warming has increased drought risk in California. PNAS 112, 3931–3936. https://doi.org/10.1073/pnas.1422385112

Dupras, J., Alam, M., Reveret, J.P., 2014. Economic value of Greater Montreal's non-market ecosystem services in a land use management and planning perspective. Can. Geogr. 1–14. https://doi.org/10.1111/cag.12138

Duruibe, J.O., Ogwuegbu, M.O.C., Egwurugwu, J.N., 2007. Heavy metal pollution and human biotoxic effects. Int. J. Phys. Sci. 2, 112–118. https://doi.org/10.1016/j.proenv.2011.09.146

Engelhardt, K.A.M., Ritchie, M.E., 2001. Effects of macrophyte species richness on wetland ecosystem functioning and services. Nature 411, 687–689. https://doi.org/10.1038/35079573

Farrugia, S., Hudson, M.D., Mcculloch, L., 2013. An evaluation of flood control and urban cooling ecosystem services delivered by urban green infrastructure. Int. J. Biodivers. Sci. Ecosyst. Serv. Manag. 9, 136–145. https://doi.org/10.1080/21513732.2013.782342

Fontes, R.L.F., Cox, F.R., 1998. Zinc toxicity in soybean grown at high iron concentration in nutrient solution. J. Plant Nutr. 21, 1723–1730. https://doi.org/10.1080/01904169809365517

Frouz, J., Pižl, V., Cienciala, E., Kalčík, J., 2009. Carbon storage in post-mining forest soil, the role of tree biomass and soil bioturbation. Biogeochemistry 94, 111–121. https://doi.org/10.1007/s10533-009-9313-0

Galbraith, D., Malhi, Y., Affum-Baffoe, K., Castanho, A.D.A., Doughty, C.E., et al., 2013. Residence times of woody biomass in tropical forests, Plant Ecology & Diversity. 6:1, 139-157. DOI: 10.1080/17550874.2013.770578

Gallai, N., Salles, J.M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Ge, B., Mehring, A., Levin, L.A., 2019. Urbanization alters belowground invertebrate community structure in semi-arid regions: A comparison of lawns, biofilters and sage scrub 2019. Urban and Landscape Planning. DOI: 10.1016/j.landurbplan.2019.103664

Getter, K., Rowe, D., Robertson, G., Cregg, B., Andresen, J., 2009. Carbon sequestration potential of extensive green roofs. Environ. Sci. Technol. 43, 7564–7570.

Ghani, A., Shah, A.U., Akhtar, U., 2010. Effect of Pb toxicity on growth, chlorophyll and Pb contents of two varieties of maize.pdf. Pakistan J. Nutr. 9, 887–891.

Giannini, T.C., Cordeiro, G.D., Freitas, B.M., Saraiva, A.M., Imperatriz-Fonseca, V.L., 2015. The Dependence of Crops for Pollinators and the Economic Value of Pollination in Brazil. J. Econ. Entomol. 108, 849–857. https://doi.org/10.1093/jee/tov093

Gilroy, K.L., Mccuen, R.H., 2009. Spatio-temporal effects of low impact development practices. J. Hydrol. 367, 228–236. https://doi.org/10.1016/j.jhydrol.2009.01.008

Glass, G. V, 1976. Primary, secondary, and meta-analysis of research. Am. Educ. Res. Assoc. 5, 3–8. https://doi.org/10.3102/0013189X005010003

Global Platform for Sustainable Cities, 2019. São Paolo Statement on Urban Sustainability: A call to integrate our responses to climate change, biodiversity loss, and social inequality. Accessed online 4 October 2019 at: https://www.thegpsc.org.

Goonetilleke, A., Thomas, E., Ginn, S., Gilbert, D., 2005. Understanding the role of land use in urban stormwater quality management. J. Environ. Manage. 74, 31–42. https://doi.org/10.1016/j.jenvman.2004.08.006

Graça, M., Alves, P., Gonçalves, J., Nowak, D.J., Hoehn, R., Farinha-marques, P., Cunha, M., 2018. Landscape and Urban Planning Assessing how green space types a ff ect ecosystem services delivery in Porto, Portugal. Landsc. Urban Plan. 170, 195–208. https://doi.org/10.1016/j.landurbplan.2017.10.007

Grimm, A.M., 2011. Interannual climate variability in South America: Impacts on seasonal precipitation, extreme events, and possible effects of climate change. Stoch. Environ. Res. Risk Assess. 25, 537–554. https://doi.org/10.1007/s00477-010-0420-1

Grizzetti, B., Lanzanova, D., Liquete, C., Reynaud, A., Cardoso, A.C., 2016. Assessing water ecosystem services for water resource management. Environ. Sci. Policy 61, 194–203. https://doi.org/10.1016/j.envsci.2016.04.008

Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R., Melbourne, B.A., Nicholls, A.O., Orrock, J.L., Song, D., Townshend, J.R., 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Sci. Adv. https://doi.org/10.1126/sciadv.1500052

Haines-Young, R., Potschin, M.B., 2018. Common International Classification of Ecosystem Services (CICES) V5.1 and Guidance on the Application of the Revised Structure. Available from www.cices.eu.

Hallett, C.S., Valesini, F.J., Clarke, K.R., Hoeksema, S.D., 2016. Effects of a harmful algal bloom on the community ecology, movements and spatial distributions of fishes in a microtidal estuary. Hydrobiologia 763, 267–284. https://doi.org/10.1007/s10750-015-2383-1

Hammer, T.R., Coughlin, R.E., Horn, E.T., 1974. The effect of a large urban park on real estate value. J. Am. Inst. Plann. 40, 274–277. https://doi.org/10.1080/01944367408977479

Hansford, K.M., Fonville, M., Gillingham, E.L., Coipan, E.C., Pietzsch, M.E., Krawczyk, A.I., Vaux, A.G.C., Cull, B., Sprong, H., Medlock, J.M., 2017. Ticks and Borrelia in urban and peri-urban green space habitats in a city in southern England. Ticks Tick. Borne. Dis. 8, 353–361. https://doi.org/10.1016/j.ttbdis.2016.12.009

Hatt, B.E., Fletcher, T.D., Deletic, A., 2009. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. J. Hydrol. 365, 310–321. https://doi.org/10.1016/j.jhydrol.2008.12.001

Heimann, M., Reichstein, M., 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451(17), 289-292.

Houlden, V., Weich, S., Jarvis, S., Rees, K., 2018. The relationship between greenspace and the mental wellbeing of adults : A systematic review. PLoS One 13, 1–35. https://doi.org/e0203000

Hsieh, C., Davis, A.P., 2006. Evaluation and Optimization of Bioretention Media for Treatment of Urban Storm Water Runoff. J. Environ. Eng. 131, 1521–1531.

Hung, K.-L.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R., 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. B Biol. Sci. 285, 20172140. https://doi.org/10.1098/rspb.2017.2140

Intergovernmental Panel on Climate Change, 2014. Climate Change 2014 Synthesis Report. Geneva, Switzerland. https://doi.org/10.1017/CBO9781107415324

IPBES, 2016. Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. S.G. Potts, V. L. Imperatriz-Fonseca, H. T. Ngo, J. C. Biesmeijer, T. D. Breeze, L. V. Dicks, L. A. Garibaldi, R. Hill, J. Settele, A. J. Vanbergen, M. A. Aizen, S. A. Cunningham, C. Eardley, B. M. Freitas, N. Gallai, P. G. Kevan, A. Kovács-Hostyánszki, P. K. Kwapong, J. Li, X. Li, D. J. Martins, G. Nates-Parra, J. S. Pettis, R. Rader, and B. F. Viana (eds.). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany. 36 pages.

Irvine, K.N., Devine-wright, P., Payne, S.R., Fuller, R.A., 2009. Green space, soundscape and urban sustainability: an interdisciplinary, empirical study. Local Environ. 14, 155–172. https://doi.org/10.1080/13549830802522061

Jabro, J.D., Sainju, U., Stevens, W.B., Evans, R.G., 2008. Carbon dioxide flux as affected by tillage and irrigation in soil converted from perennial forages to annual crops. J. Environ. Manage. 88, 1478–1484. https://doi.org/10.1016/j.jenvman.2007.07.012

James, P., Hart, J.E., Banay, R.F., Laden, F., 2016. Exposure to Greenness and Mortality in a Nationwide Prospective Cohort Study of Women. Environ. Heal. Perspect. 124, 1344–1352.

Jenkins, W.A., Murray, B.C., Kramer, R.A., Faulkner, S.P., 2010. Valuing ecosystem services from wetlands restoration in the Mississippi Alluvial Valley. Ecol. Econ. 69, 1051–1061. https://doi.org/10.1016/j.ecolecon.2009.11.022

Johnston, R.J., Rosenberger, R.S., 2010. Methods, trends and controversies in contemporary benefit transfer. J. Econ. Surv. 24, 479–510. https://doi.org/10.1111/j.1467-6419.2009.00592.x

Joshi, U.M., Balasubramanian, R., 2010. Characteristics and environmental mobility of trace elements in urban runoff. Chemosphere 80, 310–318. https://doi.org/10.1016/j.chemosphere.2010.03.059

Katul, G. G., Oren, R., Manzoni, S., Higgins, C., Parlange, M.B., 2012. Evapotranspiration: A process driving masstransport and energy exchange in the soil-plant-atmosphere-climate system, Rev. Geophys. 50, RG3002. doi:10.1029/2011RG000366

Kazemi, F., Beecham, S., Gibbs, J., 2011. Streetscape biodiversity and the role of bioretention swales in an Australian urban environment. Landsc. Urban Plan. 101, 139–148. https://doi.org/10.1016/j.landurbplan.2011.02.006

Kazmierczak, J., Muller, S., Nilsson, B., Postma, D., Czejak, J., Sebok, E., Jessen, S., Karan, S., Stenvig Jensen, C., Edelvang, K., Engesgaard, P., 2016. Groundwater flow and heterogeneous discharge into a seepage lake: Combined use of physical methods and hydrochemical tracers. Water Resour. Res. 52, 9109–9130. https://doi.org/10.1002/2016WR018977.Received

Klatt, B.K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., Tscharntke, T., 2014. Bee pollination improves crop quality, shelf life and commercial value. Proc. R. Soc. B Biol. Sci. 281, 20132440. https://doi.org/10.1098/rspb.2013.2440

Konrad, C., 2003. Effects of urban development on floods, USGS Fact Sheet FS-076-03. Tacoma, USA.

Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vázquez, D.P., Winfree, R., Adams, L., Crone, E.E., Greenleaf, S.S., Keitt, T.H., Klein, A.M., Regetz, J., Ricketts, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. Ecol. Lett. 10, 299–314. https://doi.org/10.1111/j.1461-0248.2007.01018.x

Kuo, F.E., Sullivan, W.C., 2001. Environment and crime in the inner city: Does vegetation reduce crime? Environ. Behav. 33, 343–367. https://doi.org/10.1177/0013916501333002

Le Coustumer, S., Fletcher, T.D., Deletic, A., Barraud, S., Poelsma, P., 2012. The influence of design parameters on clogging of stormwater biofilters: A large-scale column study. Water Res. 46, 6743–6752. https://doi.org/10.1016/j.watres.2012.01.026

Lee, J., Bang, K., 2000. Characterization of urban stormwater runoff. Water Res. 34, 1773–1780. https://doi.org/10.1016/S0043-1354(99)00325-5

Lee, B.C., Matsui, S., Shimizu, Y., Matsuda, T., Matsui, S., Shimizu, Y., Characterizations, T.M., 2005. Characterizations of the First Flush in Storm Water Runoff from an Urban Roadway. Environ. Technol. 26, 773–782. https://doi.org/10.1080/09593332608618508

Lee, A.C.K., Maheswaran, R., 2010. The health benefits of urban green spaces: a review of the evidence. J. Public Health (Bangkok). 33, 212–222. https://doi.org/10.1093/pubmed/fdq068

Lenth, J., Dugopolski, R., Quigley, M., Poresky, A., Leisenring, M., 2010. Filterra® Bioretention Systems: Technical Basis for High Flow Rate Treatment and Evaluation of Stormwater Quality Performance. Seattle, USA.

Lerner, D.N., 2002. Identifying and quantifying urban recharge : a review. Hydrogeol. J. 10, 143–152. https://doi.org/10.1007/s10040-001-0177-1

Lew, D.K., Larson, D.M., 2005. Valuing recreation and amenities at San Diego County beaches. Coast. Manag. 33, 71–86. https://doi.org/10.1080/08920750590883079

Li, H., Sharkey, L.J., Asce, M., Hunt, W.F., Asce, M., Davis, A.P., Asce, F., 2009. Mitigation of Impervious Surface Hydrology Using Bioretention in North Carolina and Maryland. J. Hydrol. Eng. 14, 407–415.

Lim, H.S., Lim, W., Hu, J.Y., Ziegler, A., Ong, S.L., 2015. Comparison of filter media materials for heavy metal removal from urban stormwater runoff using biofiltration systems. J. Environ. Manage. 147, 24–33. https://doi.org/10.1016/j.jenvman.2014.04.042

Lindt, R., Callahan, C., DeShazo, J.R., Bieber, E., 2014. Lessons Learned from Previous Projects for Green Alley Development in Los Angeles & Beyond. Los Angeles, USA.

Loarie, S., Carter, B., Hayhoe, K., McMahon, S., Moe, R., Knight, C., Ackerly, D., 2008. Climate change and the future of California's endemic flora. PLoS One 3, e2502. https://doi.org/10.1371/journal.pone.0002502

Long Beach Development Services, 2013. Low Impact Development (LID) Best Management Practices (BMP) Design Manual. Long Beach, USA.

Makepeace, D.K., Smith, D.W., Stanley, S.J., 1995. Urban Stormwater Quality: Summary of Contaminant Data. Crit. Rev. Environ. Sci. Technol. 25, 93–139. https://doi.org/10.1080/10643389509388476

Maniquiz-Redillas, M.C., Geronimo, F.K.F., Kim, L.H., 2014. Investigation on the effectiveness of pretreatment in stormwater management technologies. J. Environ. Sci. 26, 1824–1830. https://doi.org/10.1016/j.jes.2014.06.018

Maupin, M.A., Kenny, J.F., Hutson, S.S., Lovelace, J.K., Barber, N.L., Linsey, K.S., 2014. Estimated Use of Water in the United States in 2010, U.S. Geological Survey Circular 1405. https://doi.org/http://dx.doi.org/10.3133/cir1405

McBride, G.B., Stott, R., Miller, W., Bambic, D., Wuertz, S., 2013. Discharge-based QMRA for estimation of public health risks from exposure to stormwater-borne pathogens in recreational waters in the United States. Water Res. 47, 5282–5297. https://doi.org/10.1016/j.watres.2013.06.001

McPherson, E.G., 1992. Accounting for benefits and costs of urban greenspace. Landsc. Urban Plan. 22, 41–51. https://doi.org/10.1016/0169-2046(92)90006-L

McPhillips, L., Walter, M.T., 2015. Hydrologic conditions drive denitrification and greenhouse gas emissions in stormwater detention basins. Ecol. Eng. 85, 67–75. https://doi.org/10.1016/j.ecoleng.2015.10.018

Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29–43. https://doi.org/10.1046/j.1469-8137.2002.00363.x

Mehring, A.S., Hatt, B.E., Kraikittikun, D., Orelo, B.D., Rippy, M.A., Grant, S.B., Gonzalez, J.P., Jiang, S.C., Ambrose, R.F., Levin, L.A., 2016. Soil invertebrates in Australian rain gardens and their potential roles in storage and processing of nitrogen. Ecol. Eng. 97, 138–143. https://doi.org/10.1016/j.ecoleng.2016.09.005

Mehring, A.S., Levin, L.A., 2015. Potential roles of soil fauna in improving the efficiency of rain gardens used as natural stormwater treatment systems. J. Appl. Ecol. 52, 1445–1454. https://doi.org/10.1111/1365-2664.12525

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington DC. https://doi.org/10.1196/annals.1439.003

More, T.A., Stevens, T., Allen, P.G., 1988. Valuation of urban parks. Landsc. Urban Plan. 15, 139–152. https://doi.org/10.1016/0169-2046(88)90022-9

Moreno, J.L., Hernández, T., Garcia, C., 1999. Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. Biol. Fertil. Soils 28, 230–237. https://doi.org/10.1007/s003740050487

Morrison Paul, C., Johnston, W.E., and G.A.G. Frengley. 2000. Efficiency in New Zealand sheep and beef farming: The impacts of regulatory reform. The Review of Economics and Statistics. 82(2): 325-337. doi: https://doi.org/10.1162/003465300558713

Morse, R.A., Calderone, N.W., 2003. The value of honey bees as pollinators of US crops in 2000. Bee Cult. 128, 1–15.

Nowak, D.J., Greenfield, E.J., Hoehn, R.E., Lapoint, E., 2013. Carbon storage and sequestration by trees in urban and community areas of the United States. Environ. Pollut. 178, 229–236. https://doi.org/http://dx.doi.org/10.1016/j.envpol.2013.03.019

Oke, T., 1982. The energetic basis of the urban heat island. Q. J. R. Meteorol. Soc. 108, 1–24.

Oliveira, S., Andrade, H., Vaz, T., 2011. The cooling effect of green spaces as a contribution to the mitigation of urban heat: A case study in Lisbon. Build. Environ. 46, 2186–2194. https://doi.org/10.1016/j.buildenv.2011.04.034

Pan, J., Plant, J.A., Voulvoulis, N., Oates, C.J., Ihlenfeld, C., 2010. Cadmium levels in Europe: implications for human health. Environ. Geochem. Health 32, 1–12. https://doi.org/10.1007/s10653-009-9273-2

Panepinto, D., Fiore, S., Zappone, M., Genon, G., Meucci, L., 2016. Evaluation of the energy efficiency of a large wastewater treatment plant in Italy. Appl. Energy 161, 404–411. https://doi.org/10.1016/j.apenergy.2015.10.027

Pascual, U., Perrings, C., 2007. Developing incentives and economic mechanisms for in situ biodiversity conservation in agricultural landscapes. Agric. Ecosyst. Environ. 121, 256–268. https://doi.org/10.1016/j.agee.2006.12.025

Paule-Mercado, M.A., Ventura, J.S., Memon, S.A., Jahng, D., Kang, J.H., Lee, C.H., 2016. Monitoring and predicting the fecal indicator bacteria concentrations from agricultural, mixed land use and urban stormwater runoff. Sci. Total Environ. 550, 1171–1181. https://doi.org/10.1016/j.scitotenv.2016.01.026

Payne, E.G.I., Fletcher, T.D., Russell, D.G., Grace, M.R., Cavagnaro, T.R., Evrard, V., Deletic, A., Hatt, B.E., Cook, P.L.M., 2014. Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofiltration systems. PLoS One 9. https://doi.org/10.1371/journal.pone.0090890

Payne, E.G.I., Pham, T., Deletic, A., Hatt, B.E., Cook, P.L.M., Fletcher, T.D., 2018. Which species? A decision-support tool to guide plant selection in stormwater bio fi lters. Adv. Water Resour. 113, 86–99. https://doi.org/10.1016/j.advwatres.2017.12.022

Pearce, D., Atkinson, G., Mourato, S., 2006. Cost-Benefit Analysis and the Environment, OECD. https://doi.org/10.1787/9789264010055-en

Pendleton, L., Kildow, J., 2006. The Non-Market Value of California's Beaches Shore and Beach, Journal of the American Shore and Beach Preservation Association. 74(2): 34-37.

Poff, N.L., Zimmerman, J.K.H., 2010. Ecological responses to altered flow regimes: A literature review to inform the science and management of environmental flows. Freshw. Biol. 55, 194–205. https://doi.org/10.1111/j.1365-2427.2009.02272.x

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: Trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Ramsar Convention Secretariat, 2016. An Introduction to the Ramsar Convention on Wetlands, 7th ed. Gland, Switzerland.

Read, E.M., Strecker, E., 2013. Report of Monitoring, Operation and Maintenance. Edith Read and Associates and Geosyntec Consultants.

Reitz, M., Sanford, W.E., Senay, G.B., Cazenas, J., 2017. Annual Estimates of Recharge, Quick-Flow Runoff, and Evapotranspiration for the Contiguous U.S. Using Empirical Regression Equations. J. Am. Water Resour. Assoc. 53, 961–983. https://doi.org/10.1111/1752-1688.12546

Scanlon, B.R., Healy, R.W., Cook, P.G., 2002. Choosing appropriate techniques for quantifying groundwater recharge. Hydrogeol. J. 10, 18–39. https://doi.org/10.1007/s10040-0010176-2

Schmidt, P., Sickles, R.C., 1984. Production Frontiers and Panel Data. Journal of Business & Economic Statistics. 2:4, 367-374

Science for Environment Policy, 2015. Ecosystem Services and Biodiversity. https://doi.org/10.2779/57695

Seager, R., Ting, M., Li, C., Naik, N., Cook, B., Nakamura, J., Liu, H., 2013. Projections of declining surface-water availability for the southwestern United States. Nat. Clim. Chang. 3, 482–486. https://doi.org/10.1038/nclimate1787

Shanker, A.K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S., 2005. Chromium toxicity in plants. Environ. Int. 31, 739–753. https://doi.org/10.1016/j.envint.2005.02.003

Sharkey, L.J., 2006. The performance of bioretention areas in North Caroline: A study of water quality, water quantity, and soil media. North Caroline State University.

Shashua-Bar, L., Pearlmutter, D., Erell, E., 2009. The cooling efficiency of urban landscape strategies in a hot dry climate. Landsc. Urban Plan. 92, 179–186. https://doi.org/10.1016/j.landurbplan.2009.04.005

Sheng, J., Wilson, Æ.J.P., 2009. Watershed urbanization and changing flood behavior across the Los Angeles metropolitan region 41–57. https://doi.org/10.1007/s11069-008-9241-7

Shipitalo, M.J., Bayon, R. Le, 2004. Quantifying the effect of Earthworms on Soil Aggregation and Porosity. Earthworm Ecol. 10, 183–200.

Shwartz, A., Turbé, A., Simon, L., Julliard, R., 2014. Enhancing urban biodiversity and its influence on city-dwellers: An experiment. Biol. Conserv. 171, 82–90. https://doi.org/10.1016/j.biocon.2014.01.009

Sileshi, R., Pitt, R.E., Clark, S.E., 2019. Statistical analyses of flow rates of stormwater treatment bioretention media. Water Environ. Res. 1–11. https://doi.org/10.1002/wer.1129

Simmers, I., 1997. Groundwater recharge principles , problems and developments, in: Recharge of Phreatic Aquifers in (Semi-)Arid Areas.

The Low Impact Development Center, 2010. Low Impact Development Manual for Southern California. https://doi.org/10.1017/CBO9781107415324.004

Thrupp, L., 2000. Linking Agricultural Biodiversity and Food Security: The Valuable Role of Sustainable Agriculture. Int. Aff. 76, 265–281. https://doi.org/10.1111/1468-2346.00133

Tingsanchali, T., 2012. Urban flood disaster management. Procedia Eng. 32, 25–37. https://doi.org/10.1016/j.proeng.2012.01.1233

Todd, D.K., 1959. Annotated bibliography on artificial recharge of ground water through 1954. Washington DC.

Tubau, I., Vázquez-suñé, E., Carrera, J., Valhondo, C., Criollo, R., 2017. Quantification of groundwater recharge in urban environments. Sci. Total Environ. 592, 391–402. https://doi.org/10.1016/j.scitotenv.2017.03.118

Tzoulas, K., Korpela, K., Venn, S., Yli-pelkonen, V., Ka, A., Niemela, J., James, P., 2007. Promoting ecosystem and human health in urban areas using Green Infrastructure : A literature review. Landsc. Urban Plan. 81, 167–178. https://doi.org/10.1016/j.landurbplan.2007.02.001

U.S. Census Bureau, 2010. Population Density Data. Retrieved from https://www.census.gov/data/tables/2010/dec/density-data-text.html

U.S. Environmental Protection Agency, 2000. Low Impact Development (LID): A Literature Review.

U.S. Environmental Protection Agency, 2012a. Benefits of Low Impact Development: How LID Can Protect Your Community's Resources. Washington DC.

U.S. Environmental Protection Agency, 2012b. Aesthetics of Low Impact Development: LID Technologies Can Benefit Your Community's Visual Environment. Washington DC.

U.S. Environmental Protection Agency, 2016. Social Cost of Carbon.

Van den Berg, A.E., Maas, J., Verheij, R.A., Groenewegen, P.P., 2010. Green space as a buffer between stressful life events and health. Social Science & Medicine. 70, 1203-1210. DOI: 10.1016/j.socscimed.2010.01.002

Van Puijenbroek, M.E.B., Teichmann, C., Mejidam, N., Oliveras, I., Berendse, F., Limpens, J., 2017. Doess salt stress constrain spatial distribution of dune building grasses *Ammophila arenia* and *Elytrichia juncea* on the beach? Ecology and Evolution, 7, 7290-7303. DOI: 10.1002/ece3.3244

Van Renterghem, T., Hornikx, M., Forssen, J., Botteldooren, D., 2013. The potential of building envelope greening to achieve quietness. Build. Environ. 61, 34–44. https://doi.org/10.1016/j.buildenv.2012.12.001

Vázquez-Suñé, E., Carrera, J., Tubau, I., Sánchez-Vila, X., Soler, A., 2010. An approach to identify urban groundwater recharge. Hydro Earth Syst Sci, 14:2085-209. https://doi.org/10.5194/hess-14-2085-2010

Veitch, J., Ball, K., Crawford, D., Abbott, G., Salmon, J., 2012. Park improvements and park activity: a natural experiment. Am. J. Prev. Med. 42, 616–619.

Walsh, C.J., Roy, A.H., Feminella, J.W., Cottingham, P.D., Groffman, P.M., Ii, R.P.M., Ii, R.A.P.M.O., 2005. The urban stream syndrome: current knowledge and the search for a cure. J. North Am. Benthol. Soc. 24, 706–723.

Walsh, C.J., Fletcher, T.D., Burns, M.J., 2012. Urban Stormwater Runoff: A New Class of Environmental Flow Problem. PLoS One 7, e45814. https://doi.org/10.1371/journal.pone.0045814

Water Quality, Supply, and Infrastructure Improvement Act of 2014, AB-1471. 2014.

Watson, K.B., Ricketts, T., Galford, G., Polasky, S., O'Niel-Dunne, J., 2016. Quantifying flood mitigation services: The economic value of Otter Creek wetlands and floodplains to Middlebury, VT. Ecol. Econ. 130, 16–24. https://doi.org/10.1016/j.ecolecon.2016.05.015

Wheater, H., Evans, E., 2009. Land use, water management and future flood risk. Land use policy 26, S251–S264. https://doi.org/10.1016/j.landusepol.2009.08.019

Whitehorn, P.R., Connor, S.O., Wackers, F.L., Goul-, D., 2012. Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. Sciencexpress 10–13. https://doi.org/10.1111/j.1365-2664.2009.01759.x

Winfree, R., Gross, B.J., Kremen, C., 2011. Valuing pollination services to agriculture. Ecol. Econ. 71, 80–88. https://doi.org/10.1016/j.ecolecon.2011.08.001

Wolch, J.R., Byrne, J., Newell, J.P., 2014. Urban green space, public health, and environmental justice: The challenge of making cities "just green enough." Landsc. Urban Plan. 125, 234–244. https://doi.org/10.1016/j.landurbplan.2014.01.017

Wuana, R.A., Okieimen, F.E., 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecol. 2011. https://doi.org/10.5402/2011/402647

Yang, Y.Y., Toor, G.S., 2017. Sources and mechanisms of nitrate and orthophosphate transport in urban stormwater runoff from residential catchments. Water Res. 112, 176–184. https://doi.org/10.1016/j.watres.2017.01.039

Yruela, I., 2005. Copper in plants. Brazilian J. Plant Physiol. 17, 145–156. https://doi.org/10.1590/S1677-04202005000100012

Zhang, Z., Rengel, Z., Liaghati, T., Antoniette, T., Meney, K., 2011. Influence of plant species and submerged zone with carbon addition on nutrient removal in stormwater biofilter. Ecol. Eng. 37, 1833–1841. https://doi.org/10.1016/j.ecoleng.2011.06.016

Zinger, Y., Blecken, G.T., Fletcher, T.D., Viklander, M., Deletić, A., 2013. Optimising nitrogen removal in existing stormwater biofilters: Benefits and tradeoffs of a retrofitted saturated zone. Ecol. Eng. 51, 75–82. https://doi.org/10.1016/j.ecoleng.2012.12.007

Chapter 6: Carbon efficiency of managed urban landscapes in southern California

Jennifer T. Le, Andrew S. Mehring, Jennifer Gonzalez, Sunyoung Park, Jessica Kurylo, Lisa A. Levin

Abstract

Climate-regulating services are of growing interest as greenhouse gas concentrations continue to increase in the atmosphere; urban areas contribute sixty percent of this increase. Here, we investigated ecosystem services related to climate by examining carbon dioxide and methane uptake and emissions within bioretention systems (biofilters and bioswales in this study) in relation to other urban landscapes (grass lawns, horticultural gardens, and natural coastal sage scrub) in San Diego, California. We found net emissions of greenhouse gasses in all sampled urban landscapes; none functioned as urban sinks of atmospheric carbon. We used data envelopment analysis to reveal bioretention systems and natural coastal sage scrub as more carbon efficient, i.e. emit less carbon given environmental conditions, than grass lawns and horticultural gardens. This operations research approach also identified likely drivers of efficiency among soil characteristics (i.e. soil moisture, total carbon, and total nitrogen) for each sampling site. Based on our results, we provide urban planning and management suggestions to reduce urban carbon emissions, including the use of native vegetation which can provide both carbon and water gains. Understanding urban climate-regulating services, such as those related to carbon, is necessary for holistic decision-making to ensure a sustainable future faced with climate change.

Introduction

Although ecosystem services are benefits associated with natural processes (MEA, 2005), they can also be provided by human-made systems (Chapter 5). In the urban environment, green spaces contribute to a host of cultural, provisioning, and regulating services. For example, nature spaces and parks provide recreation areas and educational opportunities (Andersson et al., 2014). Gardens have the potential to produce fruits, vegetables, and herbs. Perhaps most important are urban regulating services, such as stormwater infiltration (Hatt et al., 2009), air and water pollutant removal (Jim & Chen, 2008; Payne et al., 2014), micro-climate regulation (Wang et al., 2017a), and carbon sequestration and storage.

Climate regulation, including carbon sequestration and storage, is an increasingly important ecosystem service in the face of anthropogenic climate change (Franklin et al., 2016). Carbon dioxide is the largest contributor to atmospheric greenhouse gas emissions, increasing by 40% since pre-industrialization (IPCC, 2014). Methane is another potent greenhouse gas which has increased by 150% due to fossil fuel burning and increased wetland emissions due to anthropogenic radiative forcing (IPCC, 2014; Bousquet et al., 2006). In addition to burning fossil fuels, land-use changes are another important source of atmospheric carbon emissions (Arneth et al., 2017). Terrestrial primary production and respiration are an integral part of the global carbon cycle. As plants photosynthesize, they fix atmospheric carbon into organic carbon that can be stored as biomass. However, development and urbanization have replaced native vegetation with cement, asphalt, and managed landscapes, decreasing the terrestrial biome's capacity to capture and store carbon (Delphin et al., 2016). Urbanization has also introduced new carbon pools in buildings, landfills, and urban vegetation (Churkina et al., 2016).

Carbon sequestration and storage have been measured in urban areas. Urban lawns in southern California are estimated to sequester an average of 0.14 kg C m^{-2} yr^{-1} and store 1.2 kg C m^{-2} in the top 20 cm of soil, which is not enough to offset emissions that are associated with their maintenance (Townsend-Small & Czimczik, 2010). Nowak et al. (2013) estimated carbon sequestration and storage in U.S. urban trees to be 0.205 kg C m^{-2} yr^{-1} and 7.69 kg C m^{-2}, respectively. In China, urban green infrastructure (e.g. parks, gardens, green roofs, forests, and wilderness areas) is estimated to sequester 0.216 kg C m^{-2} yr^{-1} and store 18.7 million tons C nationally (Chen, 2015). Although there is some evidence that urban vegetation can enhance carbon sequestration, vegetation characteristics and soil respiration can affect whether they are net carbon sinks or sources (Cameron et al., 2012; Velazquez et al., 2016). Turf grass, which has high aesthetic value, was found to be a net source of carbon dioxide in Russia (Shchepeleva et al., 2017). According to the United Nations, urban areas produce 60% of global greenhouse gas emissions (UN Habitat, 2014). As a result, urban planners and managers are coming up with innovative ways to reduce emissions and to seek sequestration options (van Doren et al., 2018; Wu et al., 2018).

An increasingly popular urban land use is biorentention (i.e. biofilters and bioswales), often employed as green infrastructure strategies for managing stormwater runoff. They provide a host of stormwater regulating services, such as slowing runoff flow, reducing runoff volume, and removing contaminants from runoff (Hatt et al., 2009; Payne et al., 2014), which are common urban water issues. Bioretention systems are becoming more widespread in urban areas that experience periodic water issues (Ambrose & Winfrey, 2015). Southern California is prone to droughts, which are predicted to increase in frequency and magnitude due to climate change (Diffenbaugh et al., 2015), and flooding during storm events. The majority of its human

population is concentrated near the coast where runoff has a more direct route to the ocean and potential for pollutants to degrade coastal environmental quality. The federal government passed the Clean Water Act in 1972, in part, to address stormwater runoff issues. In San Diego, the Regional Water Quality Control Board employs National Pollutant Discharge Elimination System permits for Multiple Separate Storm Sewer Systems (MS4) to comply with the rules and requirements of the Clean Water Act (Order No. R9-2013-0001; Aguirre, 2015). Biofilters can be an integral component of MS4 compliance and are growing in popularity due to their multiple potential benefits to the urban landscape.

Bioretention systems also have the potential to contribute to climate regulation due to their utilization of vegetation and soil media. However, whether biofilters and bioswales are net carbon sinks or sources is still uncertain. Unlike urban green spaces such as grass lawns or horticultural gardens, biofilters and bioswales receive water mainly from storm events rather than regular irrigation. Soil moisture and temperature have been shown to be positively correlated with soil respiration (Smith et al., 2003). Additionally, increasing soil moisture can inhibit aeration to create anoxic conditions and thus methane release. Pruning and mowing of maintained landscapes releases plant biomass for microbial breakdown and release of carbon dioxide. Because biofilters and bioswales do not often receive the same maintenance as other managed land uses, they may be better candidates for urban carbon capture and storage.

Here, we adopt a data envelopment analysis (DEA) approach. DEA is an operations research approach that creates “best-practice frontiers” (Charnes et al., 1978; Aigner, Lovell, & Schmidt, 1977). DEA is often used to inform decisions regarding banking, health care, agriculture, transportation, and education (Liu et al., 2013). Efficiency is defined as the maximum ratio of the sum of weighted outputs to the sum of weighted inputs. In the context of

this study, efficiency translates into how effectively environmental variables, such as soil characteristics, are converted into carbon uptake. From a management perspective, it is important to assess if biofilters are under- or outperforming other urban green spaces with respect to carbon services in order to develop holistic rules, regulations, and policies regarding urban stormwater runoff. Additionally, urban green spaces provide a unique opportunity to manipulate ecosystem services and promote innovative design options because they can be designed to meet specified criteria. This is also why we consider both outputs and inputs because, in places like southern California that experience periodic drought, it may not be practical or beneficial to increase bioretention irrigation in order to capture more carbon.

This paper represents the first step towards quantifying carbon services provided by biofilters at the University of California San Diego (UCSD). The paper objectives are (1) to determine if there is net uptake or emission of atmospheric carbon by biofilters, (2) to compare biofilters to other urban greenspaces (grass lawns, horticultural gardens, and natural coastal sage scrub) with respect to "carbon efficiency", and (3) to consider design tradeoffs that may enhance carbon services. We hypothesize that biofilters will be an intermediate performer with respect to carbon fluxes between natural sites, and managed lawns and gardens. Lawns and gardens receive regular irrigation and maintenance, whereas natural sites do not. A biofilter represents the intermediate that receives periodic irrigation and maintenance.

Methods

Location

UCSD is located in La Jolla, California, USA, characterized by a Mediterranean climate with warm, dry summers and cool, moist winters. Average annual rainfall is approximately 10.4

inches, falling mostly between December and March (U.S. Climate Data, 2019). Sampling locations were on and adjacent to UCSD (from north to south): Sanford Consortium for Regenerative Medicine and Salk Institute for Biological Studies (SCSI), the Structural & Materials Engineering building (SME), the Altman Clinical & Translational Research Institute (ACTRI), Osler Lane, and Scripps Institution of Oceanography (SIO) (Figure 6.1). These locations were chosen based on presence of all land uses in close geographical proximity to each other in order to minimize environmental variation. The farthest distance between land uses within a sampling location is approximately 470 m between the SME garden and natural sites.

Land uses

Four land uses were included in this study: stormwater biofilters, grass lawns, horticultural gardens, and natural coastal sage scrub (Figure 6.2). The biofilters are irrigated, periodically pruned, and vary in their size, vegetation, and placement (parking lots [SCSI, Osler], adjacent to buildings [SME, ACTRI], or other [SIO]). Lawns and gardens are regularly irrigated and maintained (i.e. mowing, pruning, weed removal, replanting). Within the lawns, *Pennisetum clandestinum* (kikuyu) and *Cynodon dactylon* (Bermuda) were the most common and dominant grass species (Kurylo, unpublished). Gardens ranged in structure and composition, from areas with ornamental species to native vegetation. Natural sites were considered to be representative of coastal sage scrub vegetation, and receive no regular management or maintenance. For this study, "site" refers to one land use at one location, e.g. SIO biofilter, ACTRI garden.

Carbon dioxide and methane measurements

At each site, two 50 cm-by-50 cm aluminum base frames were inserted flush into the ground to create a seal (Figure 6.3A). A hatchet was used to make slots into the soil to minimize disturbance during base installation. Installed bases were allowed to rest for at least one week before gas measurements were taken. In our study, treatment will refer to vegetation: one base enclosed vegetation characteristic of the site (or land use) and one base enclosed a relatively unvegetated space. However, not all unvegetated bases remained so over the course of our study. Routine watering and pruning continued as scheduled.

Gas measurements were taken in August 2018 (summer) and March 2019 (winter). A 50 cm-tall chamber was mounted onto the base to create a closed cell that did not allow gas exchange and minimized solar radiation shading. We used a Los Gatos Research Ultraportable Greenhouse Gas Analyzer to measure concentrations of carbon dioxide, methane, and water vapor (used as a correction factor). Measurements were taken between 10AM and 2PM (local time) on sunny, clear days to capture plant activity during time periods that receive the most solar radiation. Shade cloths (up to five, including a blackout cover) were used to manipulate light levels. Sixty to ninety-second incubation measurements were taken at each base per light level. One blackout measurement was taken at the beginning and end. The incubation times were chosen to be long enough to observe changes in carbon fluxes, but brief in order to minimize increases in air temperature and carbon dioxide limitation. A Li-Cor LI-190R photosynthetically-active radiation (PAR) sensor, two Stevens Hydra-Probe II soil moisture and temperature probes, and an Onset HOBO air temperature and relative humidity logger were also used during gas measurements to collect concurrent associated data (Figure 6.3B, 3C).

Soil sampling

In April 2019 after winter GHG sampling, soil samples were taken either directly adjacent to or as close as possible to each base. 238 samples were taken to a depth of 15 cm: 118 for determination of soil organic matter (SOM) and 120 for total carbon and total nitrogen. Samples were vertically fractioned from 0-5 cm (shallow) and 5-10 cm (deep) for SOM analysis, and from 0-1 cm (shallow) and 9-10 cm (deep) for carbon and nitrogen analysis. Roots, organisms, and other debris were removed following collection. All soils were oven-dried at 60°C for at least 72 hours until completely dry. Loss-on-ignition (LOI) was used to determine SOM (Ball, 1964; Heiri et al., 2001). We used 5 g samples that were combusted at 550°C for 4 hours. Carbon and nitrogen samples, homogenized and weighing between 0.05-0.10 g, were analyzed on a Costech elemental analyzer (Washington State University).

Plant sampling

Plants contribute to measured carbon dioxide fluxes through uptake during photosynthesis and emission during respiration. Both of these processes are dependent on biomass, which influences resource acquisition for growth and reproduction (Evans, 2013), thus necessitating a measure of aboveground biomass. Each plant species within the bases was identified to the highest taxonomic resolution possible. Six to nine individuals of the same species were measured for morphological characteristics (Appendix 6A), and then harvested by cutting stems as close to the ground as possible. Fallen leaves and stems, if apparent from the individual of interest, were also collected. Plants were oven-dried at 60°C for at least 48 hours until completely dry and then weighed. For plant species with woody biomass, woody and herbaceous biomass were weighed separately. Any bases with less than 5% vegetation cover were assigned to have zero biomass.

Lawns were assumed to be maintained at the same height year-round, and therefore aboveground biomass for a given area would not change appreciably. At lawn sites, a 4.5 cm-diameter soil core was taken and grass was cut at the soil surface of the core. These clippings were then processed the same way as other plants: oven-dried at 60°C for at least 48 hours until completely dry and then weighed. Resulting biomass measurements for the core area (15.9 cm^2) was then used to extrapolate biomass within the entire base (2500 cm^2). The exception was the SCSI lawn where cores could not be taken due to aesthetic concerns. In order to estimate the biomass within SCSI lawn bases, we used the average biomass for each treatment (i.e. vegetated or unvegetated) from the other lawn sites.

Data analysis

All data analysis was done in the base package of R (version 3.5.2.) unless otherwise noted. In order to calculate daily carbon dioxide fluxes, we tested several commonly-used equations for photosynthesis-irradiance curves (Table 6.1; Jassby & Platt, 1976). The best-fit model for each combination of location, land use, vegetation treatment, and season was that with the minimal sum of squared error. Using solar radiation data from the San Diego Airport (approximately 22 km from our sampling sites; National Solar Radiation Data Base), we then estimated the carbon flux for every hour of the day in the given sampling month (e.g. August or March-April) in the most recently available year 2010 (Figure 6.4). Daily averages for the month were calculated by adding all hours and dividing by the number of days in the month. Methane rates were calculated by averaging methane fluxes from each location, land use, vegetation treatment, and season combination. They are not dependent on light but instead result from soil microbial activity (Laanbroek, 2010).

Carbon dioxide and methane fluxes were checked for normality using the Shapiro-Wilk test. Data were not able to be transformed, so we used a Kruskal-Wallace test-by-ranks and a post hoc Dunn test with a Bonferroni correction (R package 'dunn.test') to test for differences in gas fluxes among location, land use, vegetation treatment, and season. Associations between gas fluxes and soil characteristics were investigated using Spearman's rank coefficient. These same tests were used to analyze differences among soil characteristics and efficiency scores.

Data envelopment analysis

Here, efficiency is defined as a ratio between weighted outputs and weighted inputs, and "carbon efficiency" is efficiency with respect to carbon fluxes (Figure 6.5). In other words, efficiency is the rate at which inputs are converted into outputs. Radial outputs are carbon dioxide fluxes and methane fluxes (g C day^{-1} m^{-2} g $biomass^{-1}$). Inputs are soil characteristics: SOM (shallow and deep, in percent by weight), total carbon (shallow and deep, in percent by weight), total nitrogen (shallow and deep, in percent by weight), soil moisture (water fraction by volume), and soil temperature (°C). Only fluxes with all associated environmental data were used in the analysis. The objective function, in multiplier form, is as follows:

(Equation 6.1)

$$max_{u,v}\ (u_{1j}y_{1j}\ +\ u_{2j}y_{2j})$$

Subject to:

$$\left(u_{1j}y_{1i}\ +\ u_{2j}y_{2i}\right) - \sum_{i=1}^{J} v_{mj}x_{mi}\ \leq 0, i = 1, \ldots, J\ and\ m = 1, \ldots, M$$

$$\sum_{i=1}^{J} v_{mj}x_{mj}\ =\ 1$$

$$u_{mj}, v_{mj}\ >\ 0$$

Where *j* is each decision-making unit (DMU) (i.e. each combination of discretionary variables), *u* and *v* are weights for outputs *y* and inputs *x*, respectively, which are indexed by *m*. For example, v_{mj} is the weight of input x_m of DMU *j.* Solving Equation 6.1 iteratively provides ideal weights for each DMU and also identifies a best-practice frontier established by the DMUs with the largest output-input ratios. Weights or shadow prices, u_{mj} and v_{mj}, are interpreted as the relative contribution of the given variable to overall efficiency. The constraint $\sum_{i=1}^{J} v_{mj} x_{mj} = 1$ imposes convexity and generates variable returns to scale, which are most appropriate given variable and synergistic impacts of environmental conditions on carbon fluxes (e.g. Wang et al., 2008).

We specify an input orientation, i.e. outputs are kept constant while inputs change to increase efficiency, because environmental managers have more direct control over inputs, i.e. soil characteristics, than outputs. Efficiency scores range between 1 for the most efficient and 0 for the least efficient. A full frontier was estimated using all combinations of location, land use, vegetation treatment, and season as individual DMUs (R package 'rDEA' and "Benchmarking'). Additional frontiers were estimated for vegetated bases, unvegetated bases, summer measurements, and winter measurements. Differences in efficiency scores were assessed as described above whereas differences in frontiers were assessed with a Kolmogorov-Smirnov test. Tobit models, which account for the censoring of efficiency from 0 to 1, were used to determine which factors affect efficiency (R package 'AER').

Allometric models for aboveground plant biomass

We used direct measurements of plant morphology and biomass to develop allometric models for aboveground biomass. Biomass data were tested for normality using the Shapiro-

Wilk test. For plant species with normally distributed biomass data, linear regression models were built and considered sufficient when adjusted $R^2 > 0.70$. For plant species with non-normally distributed biomass data, generalized linear models were built and best specification evaluated with Akaike's Information Criterion (threshold of 2; Burnham & Anderson, 2004). Models were verified with additional individuals of the same species. In order to estimate plant biomass within a base at a given sampling time, we made the same morphological measurements used to develop the models for individuals within the bases within two weeks of gas sampling. These measurements were then used in the models to estimate biomass. For bases in which morphological measurements were not made, ImageJ was used to estimate measurements from pictures of the base. In cases where models could not be built for a plant species (e.g. due to aesthetic concerns or scarcity on the UCSD campus), we used models from species with similar growth forms to estimate biomass. Two to three additional individuals were used to validate models. Plant biomass was used to normalize each carbon flux per gram of plant biomass.

Results

Carbon dioxide

Here, positive values indicate uptake whereas negative values indicate emission. The overall median value of carbon dioxide flux was -0.198 ± 17.398 g CO_2 s^{-1} m^{-2} g $biomass^{-1}$ (Table 6.2). There was one outlier, which was defined as more than four standard deviations away from the mean and removed for subsequent analysis: the unvegetated Osler garden during winter (-119.62 g CO_2 s^{-1} m^{-2} g $biomass^{-1}$). There were no significant differences in carbon dioxide fluxes among land use, location, or season (Figure 6.6A). However, vegetated bases had

fifteen times lower carbon dioxide emissions than unvegetated bases ($X^2 = 17.51$; df = 1; $p < 0.01$). Carbon dioxide fluxes were not significantly correlated with any soil properties measured.

Methane

Overall median methane flux was $-4.78 \times 10^{-4} \pm 2.66 \times 10^{-2}$ g CH_4 s^{-1} m^{-2} g $biomass^{-1}$. There were two outliers removed: the unvegetated Osler garden during winter (-0.47 g CH_4 s^{-1} m^{-2} g $biomass^{-1}$) and the unvegetated Sanford biofilter during summer (0.23 g CH_4 s^{-1} m^{-2} g $biomass^{-1}$). Similar to the trend in carbon dioxide fluxes, there were no significant differences among location or season (Figure 6.6B). However, garden methane emissions were two orders of magnitude greater than biofilters ($X^2 = 8.18$; df = 3; $p = 0.02$). Again, vegetated bases emit two orders of magnitude less methane than unvegetated bases ($X^2 = 8.47$; df = 1; $p < 0.01$). Methane fluxes were not significantly correlated with any soil properties measured.

Data envelopment analysis

The median efficiency score of the full frontier (all combinations of location, land use, vegetation treatment, and season) was 0.998 ± 0.136 (on a scale of 0-1 with 1 being efficient), indicating a very high level of carbon efficiency. Table 6.3 summarizes all discretionary variables. Among land uses, both biofilters and natural sites were more efficient than lawns with respect to carbon services, i.e. emitting less carbon dioxide and methane ($X^2 = 30.20$; df = 3; $p < 0.02$) (Figure 6.7). Additionally, natural sites were more efficient than gardens. There were no significant differences in efficiency among locations, vegetation treatments, or seasons.

The optimal weights *u* and *v*, i.e. shadow prices, for each input and output are calculated for each combination of discretionary variables, and presented in Table 6.4 as well as Figure 6.8.

There were significant differences in weights for the following variables between lawns and natural sites: shallow carbon ($X^2 = 11.23$; df = 3; $p < 0.01$), deep carbon ($X^2 = 23.35$; df = 3; $p < 0.01$), shallow nitrogen ($X^2 = 10.37$; df = 3; $p < 0.01$), soil moisture ($X^2 = 12.31$; df = 3; $p < 0.01$), and soil temperature ($X^2 = 18.05$; df = 3; $p < 0.01$). Between biofilters and lawns, shadow prices for deep nitrogen ($X^2 = 9.92$; df = 3; $p < 0.01$), deep SOM ($X^2 = 10.87$; df = 3; $p < 0.01$), and soil temperature ($X^2 = 18.05$; df = 3; $p < 0.01$) differed. Gardens differed in deep carbon from biofilters ($X^2 = 23.35$; df = 3; $p < 0.01$) and soil moisture from natural sites ($X^2 = 12.31$; df = 3; $p = 0.02$). Gardens and lawns significantly differed in shadow prices for deep carbon ($X^2 = 23.35$; df = 3; $p < 0.01$) and soil temperature ($X^2 = 26.75$; df = 3; $p < 0.01$). There were also significant differences in shadow prices for soil moisture ($X^2 = 10.38$; df = 1; $p < 0.01$) and daily methane fluxes ($X^2 = 9.55$; df = 1; $p < 0.01$) between seasons. Between treatments, optimal weights for daily carbon dioxide fluxes were significantly different ($X^2 = 9.11$; df = 1; $p < 0.01$).

Results from our tobit model, censored at 0 and 1, identify significant factors in determining efficiency: if at the SIO or SME locations; if there is lawn land cover; amount of deep carbon; amount of deep nitrogen; and soil temperature (Table 6.5). Another frontier was calculated with only significant factors but there were no differences between the two frontiers.

Soil characteristics

There was significantly higher soil temperature in summer than winter ($X^2 = 42.9$; df = 1; $p < 0.01$) whereas winter had significantly higher soil moisture than summer ($X^2 = 11.59$; df = 2; $p < 0.01$). Lawns and gardens had approximately six times greater soil moisture than natural sites ($X^2 = 26.75$; df = 3; $p < 0.01$). Please refer to Table 6.6 for a summary of SOM, total carbon, and total nitrogen. Lawns had the most shallow SOM ($X^2 = 43.62$; df = 3; $p < 0.01$), whereas both

lawns and gardens had higher deep SOM than biofilters and natural sites ($X^2 = 26.87$; df = 3; $p < 0.01$). There were also significant differences among locations ($X^2 = 14.96$; df = 4; $p < 0.01$) but none for vegetation treatment.

Lawns had significantly higher soil carbon than other land uses ($X^2 = 45.52$; df = 3; $p < 0.01$), but the unvegetated SME biofilter had the overall highest value for soil carbon at the soil surface (21.97%) whereas the vegetated Osler garden had the highest percent soil carbon at depth (4.32%). A similar trend holds true for nitrogen: lawns had the most nitrogen in both fractions ($X^2 = 43.91$; df = 3; $p < 0.01$). There were no significant differences in total carbon and total nitrogen between treatments, i.e. vegetation versus no vegetation.

Plant biomass

In total, 100 individuals from 16 different species were measured, collected, and used to develop the resulting models summarized in Table 6.7. The allometric models, which use body size to scale characteristics such as biomass, had high predictive power, most with adjusted $R^2 > 0.70$ using only one morphological measurement. However, there was a lot of error associated with the models, ranging from 5-350%. We were not able to produce biomass estimates for the vegetated SIO garden (due to lack of access to collection of the species) and SIO natural sites (due to clearing for fire hazard mitigation) and, therefore, they were not included in our final analysis.

Discussion

Carbon fluxes

Our results show that, while there is no net carbon uptake at any of our sites during the sampling times, vegetated sites emit less carbon dioxide and methane than unvegetated sites (Table 6.2). This may be due to aboveground biomass taking up carbon dioxide through photosynthesis. While we accounted for aboveground biomass in our carbon flux data (Table 6.7), which is a predictor of photosynthesis, the relationship between biomass and primary production can be complicated by factors such as species-specific leaf surface area and plant age (Michaletz et al., 2018). Belowground interactions with the soil community could also be driving these differences. Belowground biomass has been shown to be a significant driver of carbon fluxes (Wang et al., 2017b; Li et al., 2018). In addition to nutrient uptake and anchorage, plant roots can aerate soil, produce exudates, and contribute to litter (Eisenhauer et al., 2010; Ben-Noah & Friedman, 2018). These impacts can influence the soil community and subsequent ecological processes such as carbon and nitrogen cycling (de Deyn et al., 2008; Bardgett et al., 2014; Blouin et al., 2018). Although we did not find any significant differences in SOM, total carbon, or total nitrogen between vegetation treatments (Table 6.6), there may be other soil characteristics influenced by the presence (or species) of vegetation, such as the microbial community, which may be contributing to differences in carbon fluxes.

We sampled during seasons in which we thought our sites would experience the extremes of temperature and moisture, August being the end of summer whereas March is the end of winter, and therefore captures the spectrum of weather conditions. Although we measured significant differences in soil temperature and moisture between seasons, there was no apparent impact on carbon fluxes. Additional measurements during the spring and fall may have provided a more detailed picture of how seasonality can impact carbon fluxes.

Age may be an additional factor that contributed to our results (Kayranli et al., 2010). Our biofilter sites receive periodic maintenance (e.g. declogging, pruning and replanting of plants, replacing of media) which may disrupt carbon uptake (Moreno-Mateos et al., 2012). Older urban landscapes have also been shown to store more SOM than younger counterparts (Scharenbroch et al., 2017), contributing to carbon storage. Although initial conversion of North American prairie wetlands into agricultural lands have been shown to result in net carbon losses (Euliss et al., 2006), a study by Raciti et al. (2011) found that residential areas in Maryland (USA) that were previously agricultural land have greater potential to accumulate and store carbon and nitrogen relative to previously forested sites. Rather than agriculture, southern California urban landscapes have replaced native coastal sage scrub which have been shown to store more carbon and nitrogen than non-native grasslands (Wheeler et al., 2016).

The unusually high carbon emissions observed at the unvegetated Osler garden during winter may be due to unaccounted for mulch during biomass normalization. Mulch (in this case, wood chips) is commonly used in landscaping to prevent erosion and retain moisture (Chalker-Scott, 2007). At the Osler location, winter soil moisture was significantly higher than in the summer which may have created conditions for high levels of soil respiration and methane emissions. This may imply that unvegetated, mulched areas have the potential to be large urban sources of atmospheric carbon, especially because there are no primary producers present to offset their emissions. However, there is a tradeoff between potential for high carbon emissions and the benefits of mulch, including less erosion, moisture retention, and increased soil nutrition (Chalker-Scott, 2007).

Carbon efficiency

Our efficiency scores (i.e. technical efficiency) support our original hypothesis: biofilters and natural sites outperform lawns and gardens (only for natural sites) with respect to carbon fluxes (Table 6.3). Although there were no differences in fluxes between biofilters and other land uses, biofilters perform equally as well as natural sites. In natural sites, efficiency could be driven by physiological adaptation. Coastal sage scrub species are well-adapted to long dry seasons and can efficiently utilize nutrients (Gray, 1982). For example, during the summer, *Artemesia californica* will enter into a quasi-dormant state in which smaller, brown leaves replace green leaves (Montalvo et al., 2017). Although this means water and nutrient amendments may not be necessary (as they might be with more lush plants), there are tradeoffs with aesthetic value. However, in southern California where water demand often outweighs supply (California Department of Food & Agriculture, 2008), people may be willing to make this tradeoff for gains in both water and carbon savings. Although our biofilters did not utilize native vegetation, this may be a design option for future bioretention systems.

Additionally, unlike lawns, the biofilters in this study did not receive regular pruning and mowing. Lerman & Contosta (2019) found that increased frequency of mowing increased the biogenic release of carbon dioxide in lawns in Massachusetts, USA. Although there were no significant differences in actual carbon fluxes, maintenance may play a role in carbon efficiency by increasing emissions that would not have otherwise occurred. This highlights another potential tradeoff between carbon fluxes and aesthetic value.

The presence of a lawn negatively affected efficiency, as well as increases in deep carbon and the interaction term between soil moisture and temperature (Table 6.5). Lawns had significantly higher soil carbon than other land uses (Table 6.6), which may be a contributing factor. SOM associated with grasses and turfs has been shown to be significantly higher than in

native soils (Pouyat et al., 2009). Soil carbon can contribute to soil respiration by providing organic matter for microbial respiration. However, lawns also had significantly higher soil nitrogen, which could increase efficiency. Nitrogen is necessary for photosynthesis and plant growth, which decrease carbon emissions, but this effect seems to be outweighed by that of other variables in this case. Increases in soil moisture also positively affected efficiency, but the interaction term between moisture and temperature was negative. This implies that, for a given level of soil moisture, increases in temperature would decrease efficiency. This could be due to higher respiration rates under higher temperatures, which may be the scenario under future climate change (Carey et al., 2016).

Shadow prices

Shadow prices, i.e. optimal weights *u* and *v*, are the relative contributions of an output or input, respectively, to the overall optimum of efficiency. In other words, the ratio of shadow prices gives how much a decrease in one input would change efficiency compared to another input. They do not provide the nature of the relationship, i.e. positive or negative. The significant differences we found in shadow prices imply that efficiency scores for different locations, land uses, vegetation treatments, and seasons rely more heavily on different environmental factors (Figure 6.8). Our lawn sites had significantly higher soil carbon than other land uses (Table 6.5), perhaps to saturation so that a marginal decrease in soil carbon would not affect carbon efficiency (Ferreira et al., 2017). There may be a similar case with respect to soil moisture. However, our natural sites do not receive any nutrient or water amendments and, therefore, their carbon efficiencies may be more dependent on the amount of soil carbon or soil moisture available. The opposite case is true for soil temperature: it is weighted more heavily for lawns

than natural sites. This may be due to elevated soil moisture in lawns leading to microbial activity that can be further stimulated by increasing soil temperature (Sierra et al., 2015). Similar mechanisms may be at play between lawns and biofilters.

There are significant differences in shadow prices between seasons with respect to soil moisture and daily methane fluxes. Soil moisture was weighted higher in summer than in winter (Table 6.4). This may be because there is significantly lower soil moisture in summer (Table 6.5), which may be limiting the ability of our sites to uptake carbon. Although there were no significant differences in daily methane fluxes between seasons, they were weighted higher in winter than in summer (Table 6.4). Methane fluxes are largely dependent on soil microbial processes that may have been stimulated by higher soil moisture in winter (Laanbroek, 2010), and, as a result, contributed more to overall carbon efficiency. Daily carbon dioxide fluxes were weighted higher in vegetated sites than unvegetated sites. Although our fluxes are biomass-normalized, there may be belowground processes or indirect interactions between plants and the environment, such as microbial facilitation of nutrient uptake (Wahbi et al., 2015), that are not captured in this study.

These shadow prices can provide some management tradeoffs and recommendations to minimize urban carbon emissions. Water and nutrient amendments to biofilters and natural sites may disproportionately increase their carbon efficiency, however, with the tradeoff of irrigation and maintenance. Because soil moisture contributes less to carbon efficiency in winter than in summer, it may be recommended to re-evaluate irrigation schedules during the winter. Additionally, it may be important to monitor soil moisture during winter as it can stimulate methane emissions which have a larger contribution to efficiency relative to summer.

Management implications and conclusions

Although our study did not identify any carbon sinks, our results may be useful to urban planners and managers who are interested in reducing local carbon emissions. Lawns and gardens were significantly less efficient with respect to carbon services than natural sites and biofilters. Our results suggest that these differences are, in part, driven by soil characteristics such as soil moisture, which is a property over which landscape managers have control. In southern California, where drought is common and water is a valuable resource (Hansen et al., 2008), better management of soil moisture may provide gains in both carbon fluxes and reduced irrigation. Other suggestions to reduce urban carbon emissions include planting of native vegetation, reduced frequency of pruning and mowing, and careful use of mulch. There are already programs in place to replace water-intensive practices, such as grass lawns, with native vegetation, succulents, and other vegetation tolerant to periodic drought (California Department of Water Resources, 2018). Although these types of programs are aimed at reducing water usage, our results suggest additional gains of greater carbon efficiency.

Climate change and its impacts are a pressing issue that is integral to the health of our planet and society (e.g. as the United Nations Sustainable Development Goal 13; United Nations, 2019). California Assembley Bill 32 (the California Global Warming Solutions Act of 2006) aims to reduce greenhouse gas emissions to 1990 levels, which equates to a decrease of approximately 15%. Mitigation approaches include using alternative sources of energy, taxing fossil fuel use, dumping iron into the ocean, and other mechanisms to both reduce emissions of and sequester atmospheric carbon. Although biofilters are designed to capture and treat stormwater runoff, our study shows that they also contribute to carbon fluxes just as other urban green spaces do. We presented evidence that biofilters may not be as strong carbon emitters as

grass lawns or horticultural gardens which are common in the urban landscape. This, in addition to other services they potentially provide, may make them a more attractive stormwater strategy than previously thought. However, as biofilters become more widespread, empirical evidence of claimed co-benefits, such as carbon sequestration and storage, will be useful when making decisions regarding stormwater mitigation. The ability of urban vegetation to uptake carbon dioxide is well-documented (Demuzere et al., 2014), but few studies focus on natural stormwater treatment systems (i.e. biofilters in this case). Natural stormwater treatment systems are generally viewed from engineering and water perspectives, but rarely through the lens of carbon services. This novel perspectice can provide design and management options for carbon neutrality goals that have been set by governments (e.g. United Nations Framework Convention on Climate Change) and organizations (e.g. the University of California). As urban planners and managers strive towards sustainable development, gaining an understanding of carbon gains and efficiency is crucial to holistic, environmental decision-making.

Acknowledgements

This work was supported by the University of California Multi-campus Research Programs and Initiatives award MRP-17-455083; and the University of Southern California Sea Grant awards USC NOAA 61207781 & USC NOAA 75199714. The authors would like to thank the UC San Diego landscaping team (Chuck Morgan, Kimberly O'Connell, Mike Hogan, Mike Scarry, Sergio Bresciana, Musli Ramnabaja, Robert Hernandez), Wally Shand at the Sanford Consortium for Regenerative Medicine, Tim Ball at the Salk Institute for Biological Studies, and Patrick Bowens at Thornton Hospital for their help and support throughout the field component of this study; Lindsey Mayfield for her help preparing soil samples for analysis; Dr. Ray Lee at

Washington State University for running carbon and nitrogen analyses on our soil samples; and the rest of our MRPI team for their endless encouragement and support.

Chapter 6, in part, is in preparation for submission for publication. Le, J.T., Mehring, A.S., Gonzalez, J.P., Park, S., Kurylo, J., and L.A. Levin. Carbon efficiency of managed urban landscapes in southern California. The dissertation author was the primary investigator and author of this material.

Tables

Table 6.1. Equations that were used to find the best-fit curve for carbon dioxide flux data (g C hr^{-1} m^{-2} g $biomass^{-1}$) from Jassby & Platt (1976). α is the slope of the linear part of the curve, β is the maximum rate of photosynthesis, P is the rate of photosynthesis, and I is PAR. Count of best-fit indicates how many sets of greenhouse gas measurements the equation produced the least sum of squared error.

Mathematical equation	Equation name	Count of best-fit	Reference
$\begin{matrix} \alpha I & I \leq P_{max}/\alpha \\ P_{max} & I > P_{max}/\alpha \end{matrix}$	bilinear	53	Blackman, 1905
$\frac{\beta I}{\alpha + I}$	Michaelis-Menten	14	Baly, 1935
$\beta tanh(\frac{\alpha I}{\beta})$	tanh	3	Jassby & Platt, 1976
$\frac{\alpha\beta I}{(\beta^2 + (\alpha + I)^2)^{1/2}}$	power	0	Smith, 1936
$\alpha I e^{-\frac{\alpha I}{\beta}}$	exponential	0	Steele, 1962
$\alpha I - \frac{(\alpha I)^2}{4\beta}$	platt	2	Platt et al., 1975

Table 6.2. Summary of median carbon dioxide and methane fluxes by discretionary variables: location, land use, vegetation treatment, and season. Positive values indicate uptake whereas negative values indicate emission.

Discretionary variable	**Carbon dioxide flux** (g CO_2 day^{-1} m^{-2} g $biomass^{-1}$)	**Methane flux** (g CH_4 day^{-1} m^{-2} g $biomass^{-1}$)	**Total carbon flux** (g C day^{-1} m^{-2} g $biomass^{-1}$)
SCSI	-0.13 ± 8.25	$-2.24 \times 10^{-5} \pm 9.42 \times 10^{-3}$	-0.04 ± 2.25
SME	-0.28 ± 2.21	$-1.09 \times 10^{-3} \pm 8.01 \times 10^{-3}$	-0.08 ± 0.61
ACTRI	-0.22 ± 4.95	$-7.52 \times 10^{-4} \pm 2.18 \times 10^{-2}$	-0.06 ± 1.37
Osler	-0.07 ± 15.09	$-2.64 \times 10^{-4} \pm 6.14 \times 10^{-2}$	-0.02 ± 4.16
SIO	-0.26 ± 4.56	$-3.71 \times 10^{-4} \pm 2.23 \times 10^{-2}$	0.07 ± 1.26
Biofilter	-0.18 ± 7.96	$-1.05 \times 10^{-5} \pm 2.05 \times 10^{-2}$	-0.05 ± 2.18
Lawn	-0.20 ± 0.08	$-5.15 \times 10^{-4} \pm 5.25 \times 10^{-4}$	-0.06 ± 0.02
Garden	-0.57 ± 11.71	$-2.91 \times 10^{-3} \pm 4.94 \times 10^{-2}$	-0.16 ± 3.23
Natural	-0.07 ± 6.01	$-3.15 \times 10^{-4} \pm 1.15 \times 10^{-2}$	-0.02 ± 1.65
Vegetated	-0.05 ± 1.98	$-4.36 \times 10^{-5} \pm 5.07 \times 10^{-3}$	-0.01 ± 0.54
Unvegetated	-0.48 ± 10.28	$-1.66 \times 10^{-3} \pm 0.04 \times 10^{-2}$	-0.13 ± 2.83
Summer	-0.22 ± 8.54	$-1.05 \times 10^{-3} \pm 3.60 \times 10^{-2}$	-0.06 ± 2.36
Winter	-0.19 ± 7.02	$-2.3 \times 10^{-5} \pm 1.57 \times 10^{-2}$	-0.05 ± 1.92

Table 6.3. Median efficiency scores and standard deviations when comparing all combinations of location, land use, vegetation treatment, and season. Scores are on a scale of 0-1 with 1 being efficient.

Discretionary variable	Efficiency score
SCSI	1.00 ± 0.115
SME	0.936 ± 0.147
ACTRI	1.00 ± 0.125
Osler	0.999 ± 0.170
SIO	0.831 ± 0.109
Biofilter	1.00 ± 0.099
Lawn	0.757 ± 0.116
Garden	0.952 ± 0.140
Natural	1.00 ± 0.00
Vegetated	1.00 ± 0.136
Unvegetated	0.969 ± 0.137
Summer	0.984 ± 0.160
Winter	0.999 ± 0.071

Table 6.4. Mean optimal weight, or shadow price, and standard deviations for each input and output variable.

Variable	Shallow carbon	Deep carbon	Shallow nitrogen	Deep nitrogen	Shallow organic matter	Deep organic matter	Soil moisture	Soil temp	Daily carbon dioxide flux	Daily methane flux
SCSI	0.059 ± 0.169	0.125 ± 0.119	0.036 ± 0.109	0.906 ± 1.522	$6.231 \times 10^{-3} \pm 0.002$	0.064 ± 0.076	1.287 ± 0.897	0.016 ± 0.014	0.034 ± 0.106	0.162 ± 0.351
SME	0.014 ± 0.021	0.116 ± 0.184	0.014 ± 0.052	0.470 ± 1.244	$1.84 \times 10^{-2} \pm 6.87 \times 10^{-2}$	0.021 ± 0.052	2.395 ± 4.830	0.023 ± 0.014	0.074 ± 0.272	0.010 ± 0.022
ACTRI	0.032 ± 0.088	0.132 ± 0.00	0.403 ± 0.538	0.429 ± 1.011	0.016 ± 0.033	0.096 ± 0.114	0.943 ± 0.949	0.010 ± 0.009	0.021 ± 0.051	0.00 ± 0.00
Osler	0.006 ± 0.012	0.056 ± 0.104	0.403 ± 0.982	0.360 ± 0.768	0.002 ± 0.003	0.025 ± 0.055	3.190 ± 7.346	0.024 ± 0.016	0.067 ± 0.106	0.014 ± 0.029
SIO	$4.28 \times 10^{-2} \pm 0.014$	0.00 ± 0.132	0.370 ± 0.753	0.481 ± 0.842	$1.75 \times 10^{-2} \pm 0.005$	0.00 ± 0.00	0.717 ± 0.887	0.029 ± 0.012	0.021 ± 0.059	1.046 ± 3.219
Biofilter	0.029 ± 0.076	0.051 ± 0.082	0.324 ± 0.585	0.993 ± 1.212	0.005 ± 0.014	0.070 ± 0.092	2.293 ± 5.831	0.16 ± 0.013	0.093 ± 0.258	0.718 ± 2.548
Lawn	0.00 ± 0.00	$5.831 \times 10^{-2} \pm 0.023$	0.00 ± 0.00	$6.250 \times 10^{-4} \pm 0.003$	0.00 ± 0.00	0.00 ± 0.00	0.667 ± 0.832	0.032 ± 0.008	0.038 ± 0.0762	0.057 ± 0.117

Table 6.4. Continued

Variable	Shallow carbon	Deep carbon	Shallow nitrogen	Deep nitrogen	Shallow organic matter	Deep organic matter	Soil moisture	Soil temp	Daily carbon dioxide flux	Daily methane flux
Garden	0.047 ± 0.158	0.134 ± 0.174	0.228 ± 0.540	0.590 ± 1.337	0.003 ± 7.69 x 10^{-3}	0.045 ± 0.094	0.932 ± 1.053	0.019 ± 0.016	0.022 ± 0.062	0.028 ± 0.092
Natural	0.023 ± 0.026	0.206 ± 0.118	0.385 ± 0.887	0.597 ± 1.283	0.012 ± 0.031	0.061 ± 0.067	3.275 ± 4.918	0.0103 ± 0.009	0.015 ± 0.044	1.667 x 10^{-3} ± 5.773 x 10^{-3}
Vegetated	0.031 ± 0.114	0.104 ± 0.147	0.201 ± 0.646	0.671 ± 1.283	1.931 x 10^{-3} ± 0.007	0.040 ± 0.079	2.496 ± 5.368	0.019 ± 0.014	0.073 ± 0.196	0.026 ± 0.068
Unvegetated	0.018 ± 0.058	0.080 ± 0.117	0.247 ± 0.506	0.415 ± 0.951	0.007 ± 0.021	0.045 ± 0.077	0.942 ± 0.848	0.021 ± 0.015	0.017 ± 0.060	0.403 ± 1.866
Summer	0.039 ± 0.117	0.101 ± 0.153	0.278 ± 0.694	0.807 ± 1.363	6.085 x 10^{-3} ± 0.019	0.043 ± 0.082	2.574 ± 4.974	0.014 ± 0.010	0.039 ± 0.075	0.013 ± 0.062
Winter	6.40 x 10^{-2} ± 0.016	0.080 ± 0.102	0.156 ± 0.375	0.203 ± 0.580	2.511 x 10^{-3} ± 0.011	0.042 ± 0.072	0.603 ± 0.775	0.028 ± 0.015	0.051 ± 0.204	0.477 ± 2.00

Table 6.5. Results from a tobit model with efficiency as the dependent variable ('**' significant at $\alpha = 0.01$, '*' significant at $\alpha = 0.05$).

Variable	Coefficient	p-value
Intercept	-3.689e+02	0.021*
Site: Osler	-8.295e-02	0.135
Site: SCSI	-2.522e-03	0.963
Site: SME	-1.903e-01	<0.01**
Site: SIO	-1.141e-01	0.031*
Cover: Lawn	-1.750e-01	0.040*
Cover: Garden	-5.794e-03	0.883
Cover: Natural	4.599e-01	0.996
Season: March	-1.495e-02	0.789
Shallow carbon	7.781e-03	0.577
Shallow nitrogen	-7.806e-02	0.682
Deep carbon	-1.487e-01	<0.01**
Deep nitrogen	1.460e+00	<0.01**
Shallow organic matter	-9.214e-04	0.850
Deep organic matter	-7.349e-03	0.561
Soil moisture	1.728e+00	0.043*
Soil temperature	-5.644e-03	0.416
Daily carbon dioxide flux	-2.763e-02	0.048*
Daily methane flux	7.516e+00	0.021*
Interaction: moisture, temp	-5.889e-02	0.028*

Table 6.6. Summary of median soil organic matter (SOM), total soil carbon, and total soil nitrogen, reported in percentages by weight with standard deviations.

Variable	Shallow SOM	Deep SOM	Shallow Carbon	Deep Carbon	Shallow Nitrogen	Deep Nitrogen
SCSI	4.88 ± 6.07	3.09 ± 1.33	3.56 ± 2.87	1.27 ± 1.12	0.27 ± 0.28	0.12 ± 0.07
SME	11.42 ± 4.31	4.80 ± 2.63	6.35 ± 7.81	1.28 ± 0.95	0.45 ± 0.63	0.11 ± 0.10
ACTRI	4.55 ± 7.69	4.23 ± 1.15	1.70 ± 5.93	1.02 ± 1.20	0.11 ± 0.54	0.10 ± 0.06
Osler	6.06 ± 2.42	4.57 ±1.45	3.98 ± 2.09	1.77 ± 1.12	0.29 ± 0.23	0.15 ± 0.07
SIO	7.01 ± 6.55	6.93 ± 2.49	2.95 ± 4.05	1.92 ± 0.67	0.20 ± 0.39	0.14 ± 0.07
Biofilter	5.03 ± 3.25	4.01 ± 1.54	5.83 ± 5.37	1.67 ± 1.02	0.49 ± 0.47	0.14 ± 0.08
Lawn	17.29 ± 3.76	6.81 ± 2.79	12.35 ± 3.70	2.62 ± 0.90	1.14 ± 0.34	2.23 ± 0.08
Garden	6.33 ± 1.99	5.41 ± 1.42	3.21 ± 1.97	1.34 ± 1.29	0.30 ± 0.19	0.11 ± 0.08
Natural	5.73 ± 1.65	4.29 ± 1.28	1.94 ± 0.85	1.21 ± 0.37	0.16 ± 0.06	0.12 ± 0.03
Vegetated	6.57 ± 6.41	4.66 ± 2.67	3.27 ± 4.93	1.53 ± 1.18	0.23 ± 0.47	0.13 ± 0.09
Unvegetated	6.99 ± 5.37	4.95 ± 2.04	3.66 ± 5.81	1.71 ± 0.78	0.27 ± 0.46	0.13 ± 0.07

Table 6.7. Summary of plant biomass models.

Species	Best Predictor	Adjusted R^2	Percent Error
Aptenia cordifolia	Greatest width	0.717	39.7%
Artemisia californica	Greatest width	0.946	78.8%
Baccharis pilularis	Minimum leaf width	0.764	85.1%
Festuca mairei	Basal circumference	0.992	17.0%
Lactucca spp.	Total leaves	0.994	111.9%
Rhus integrifolia	Greatest width	0.972	164.8%
Chondropetalum tectorum	Stem density	0.918	66.3%
Solidago sp.	Height	0.953	5.4%
Osler shrub	Branch length	0.945	4.9%
Primula sp.	Height	0.703	189.1%
Salix sp.	Stem diameter	0.758	349.8%
Spurge spp.	Branches per stem	0.980	162.4%

Figures

Figure 6.1. Geographic location of all sampling locations included in this study.

Figure 6.2. Examples of land uses included in this study: (A) stormwater biofilter, (B) grass lawn, (C) horticultural garden, and (D) natural coastal sage scrub.

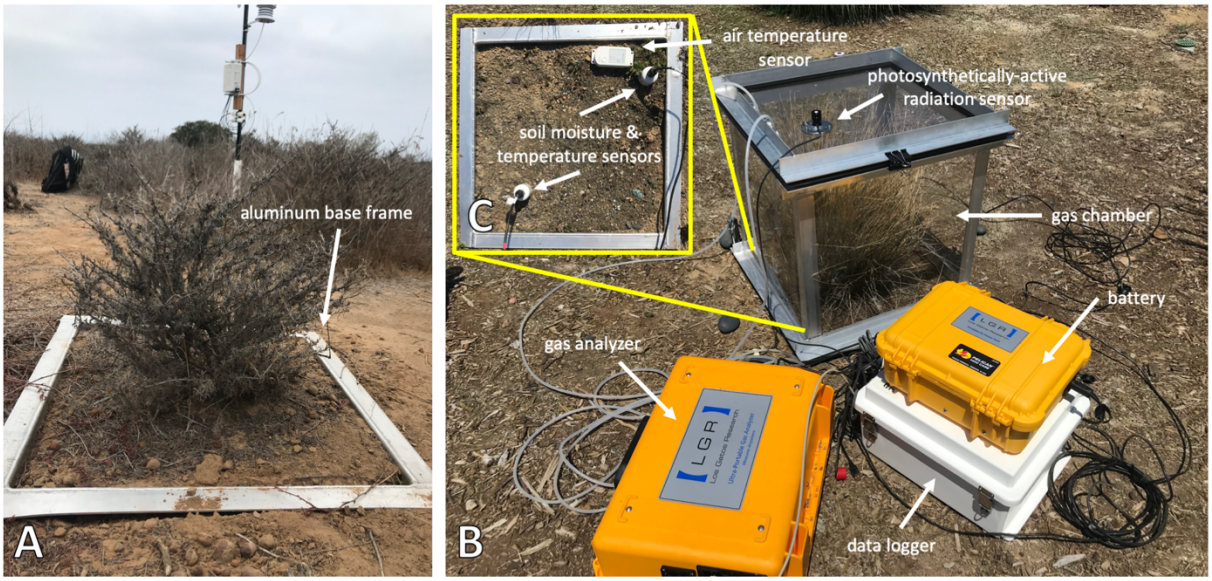

Figure 6.3. The installed aluminum base frame (A), and our field setup (B) with an inset of the top-down view (C).

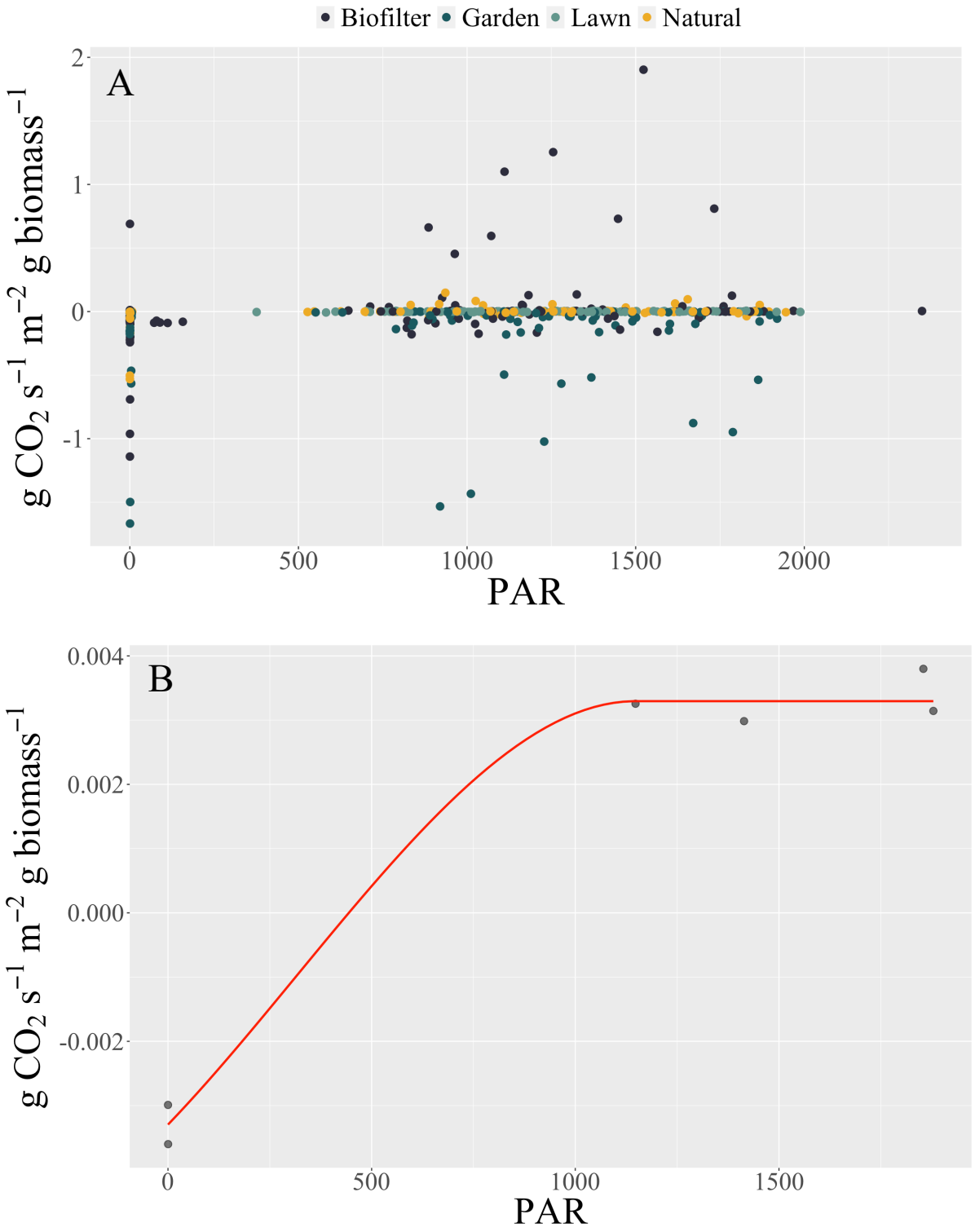

Figure 6.4. (A) Measured carbon dioxde fluxes at different values of photosynthetically-active radiation, and (B) a bilinear model fit to the measurements taken at the vegetated ACTRI biofilter during winter. Positive values indicate uptake whereas negative values indicate emissions.

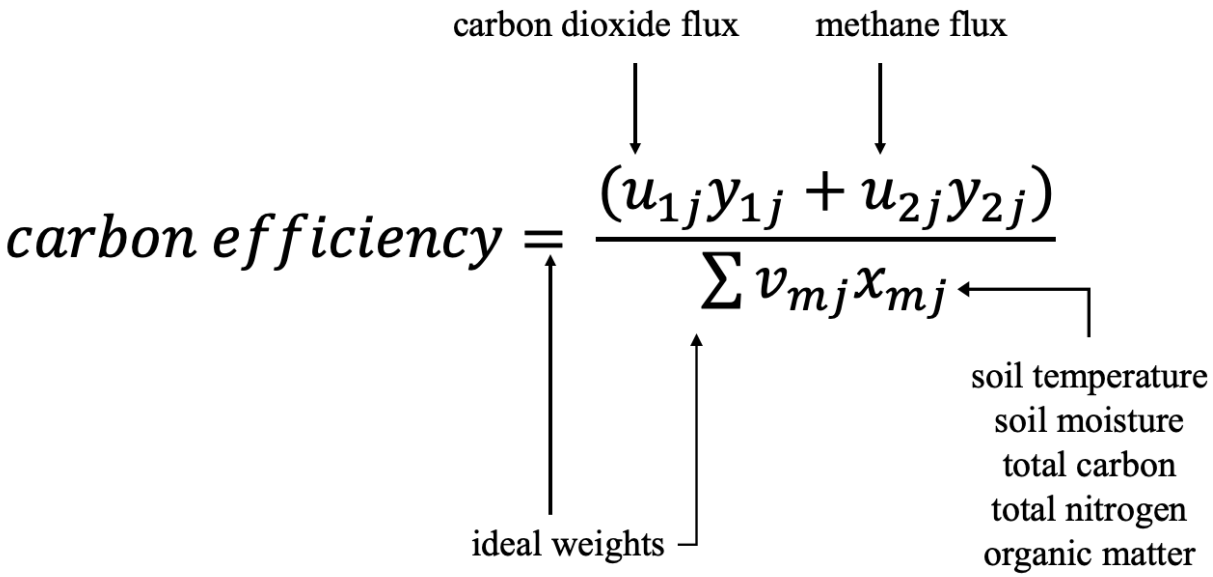

Figure 6.5. Outputs and inputs included in our definition of carbon efficiency.

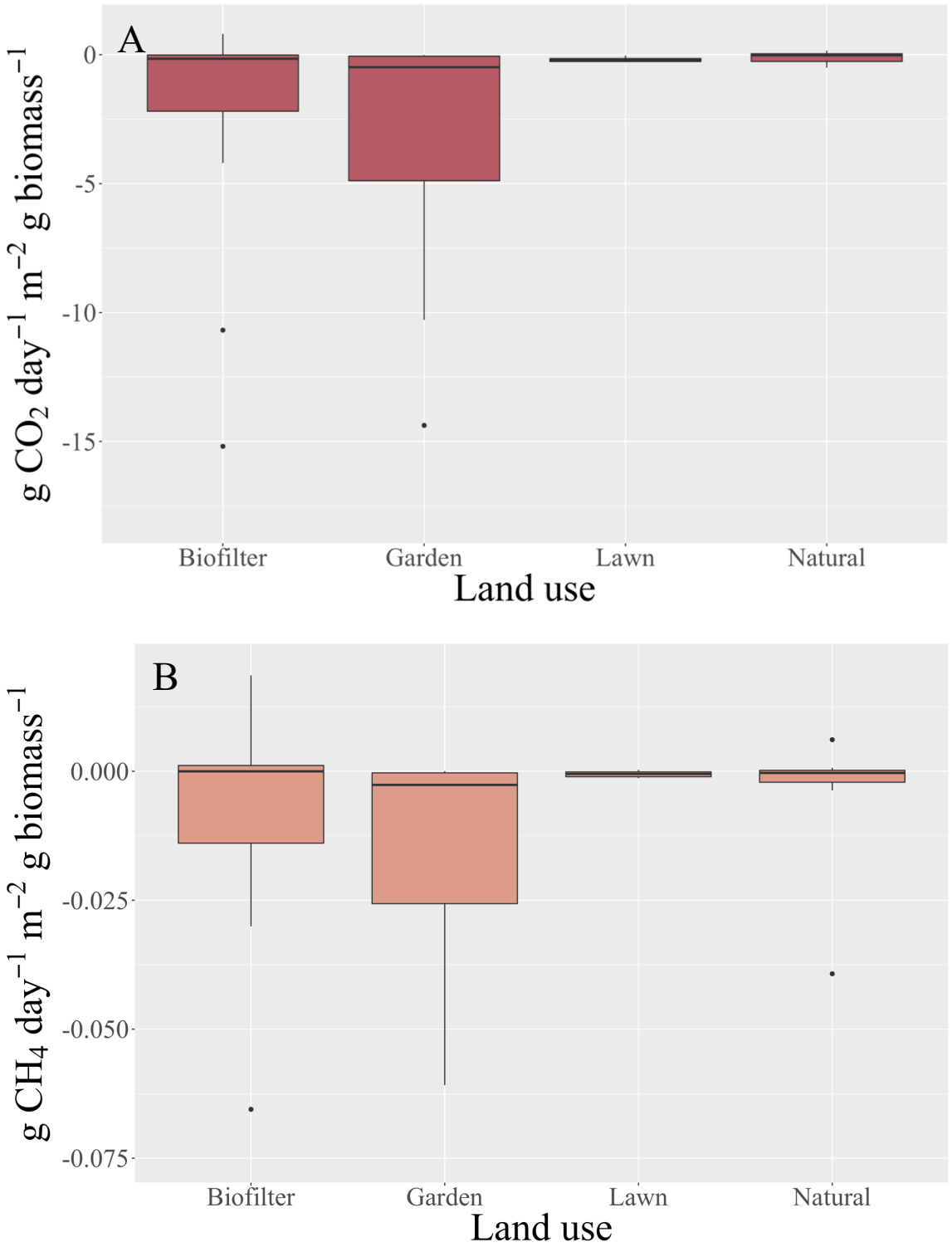

Figure 6.6. Boxplots showing the 75th quartile, median, 25th quartile, and (1.5 * interquartile range) of (A) carbon dioxide fluxes and (B) methane fluxes of each land use. Positive fluxes indicate uptake whereas negative fluxes indicate emissions.

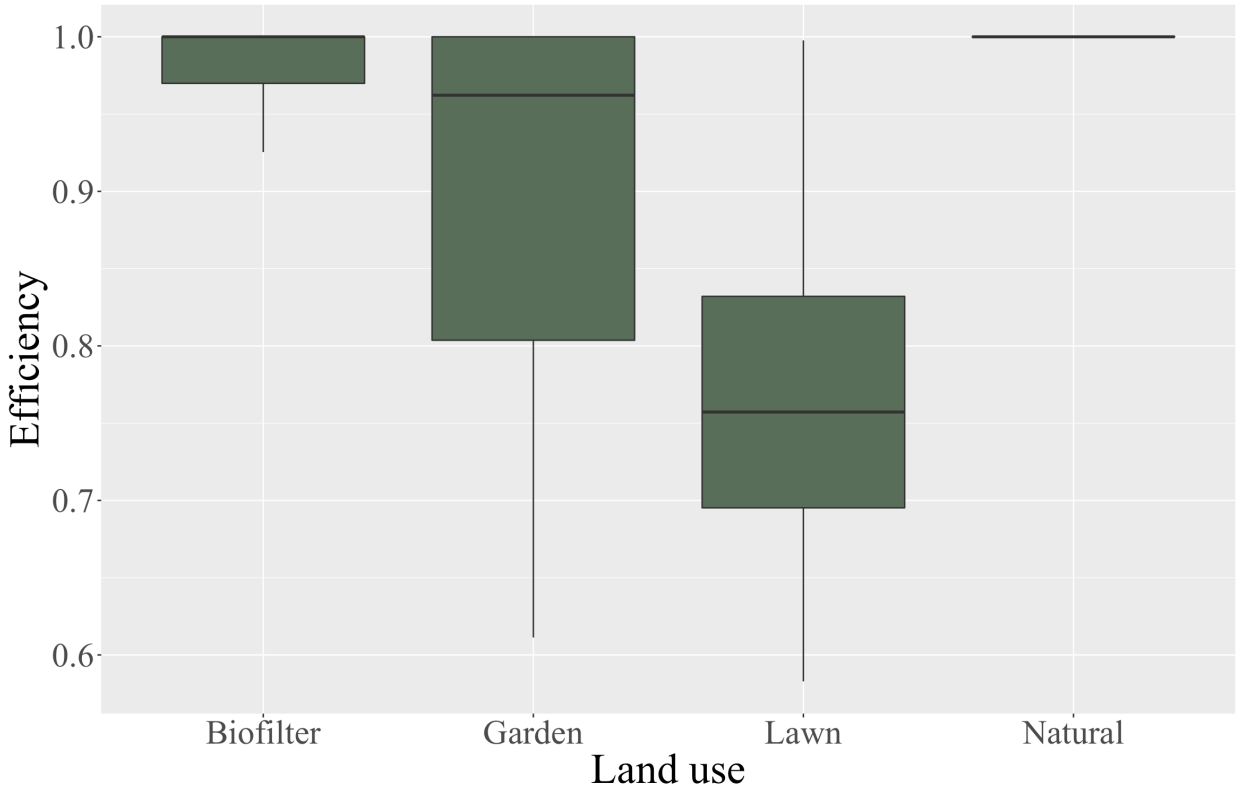

Figure 6.7. Efficiency scores of each land use. Efficiency ranges from 0 (least efficienct) to 1 (most efficient).

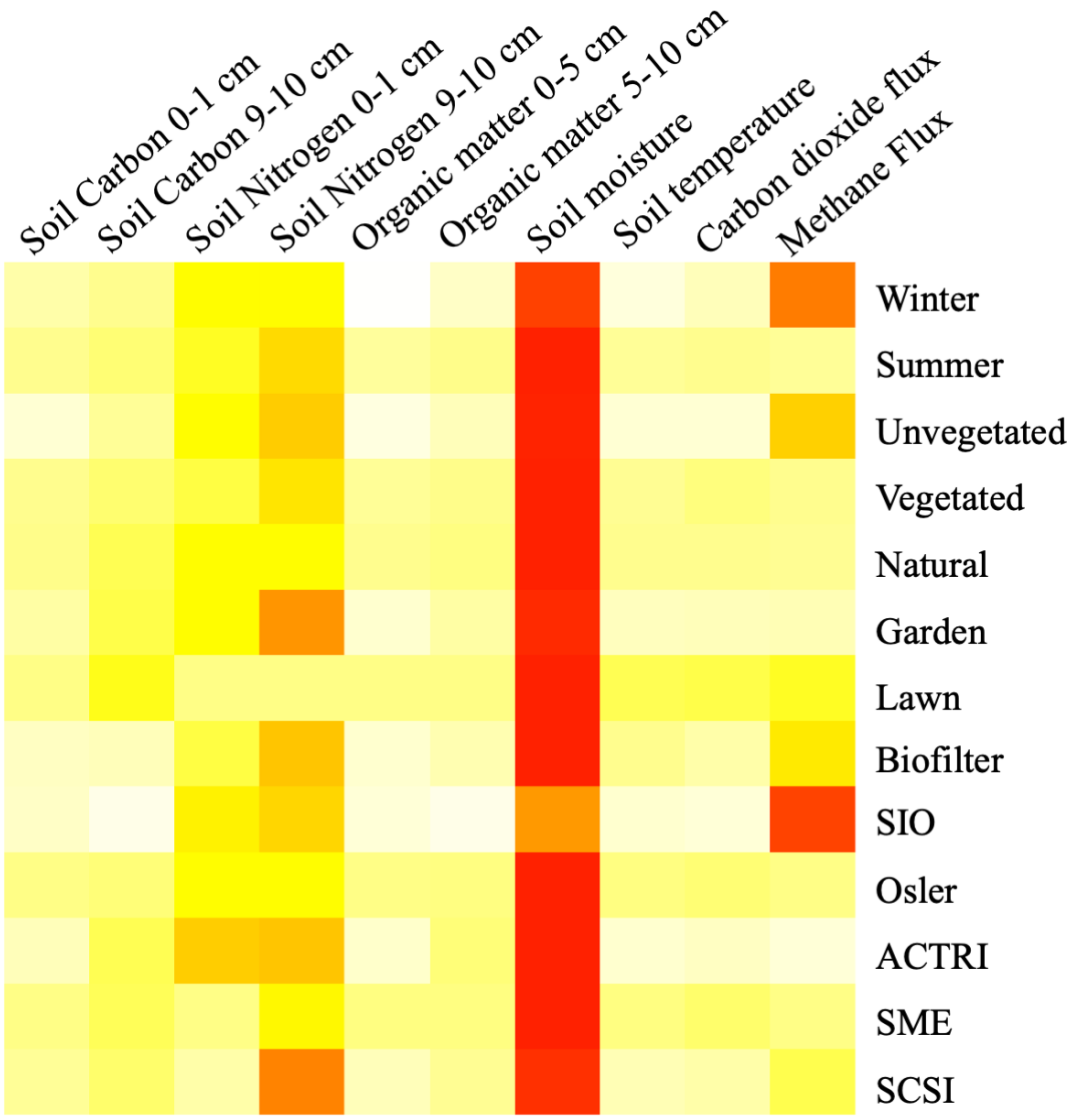

Figure 6.8. Heatmap of average relative weights (u, v) of all inputs and outputs included in our analysis. Darker colors represent higher weights.

Appendices

Appendix 7A. List of plant morphological features that were measured for incorporation into allometric biomass models. Given the large inter- and intra-species variation in morphology, not all measurements were taken for all species.

- Species to highest resolution
- Size class: large, medium, small
- Spherical densitometer for canopy cover
- Plant type / growth form: trees, shrubs, herbs (forbs or graminoids)
- Plant height & spreading/branching width (if prostrate plant, would be the same as stem/branch length)

- Branch length (to nearest cm) of the 10 longest branches (excluding leaf). Branch = anything that branches above the bottom 10% of the plant's height; in the case of trees, anything that branches from the main axis
- Diameter of 10 separate branches across a plane at mid-plant height (use calipers for this). Use the branching distance as a transect on which to evenly space points to measure
- Basal circumference (distance around stem or cluster of stems at the point closest to the ground. For spreading species, use calipers to measure stem thickness at point where plant is rooted to the ground.)
- Stem diameter
- Stem height
- Stem density (number of stems/individual, will generally be 1)
- Number of branches per stem
- Number of leaves on 5 longest branches
- Dimensions (length, width, thickness) of 3 large, 3 intermediate, and 3 small leaves
- Photos

References

Aguirre, P., 2015. Biofilters in San Diego County: A descriptive analysis of their stormwater management implications, the water regime's role, and the way forward. University of California, San Diego.

Aigner, D., Lovell, C.A.K., Schmidt, P., 1977. Formulation and estimation of stochastic frontier production function models. J. Econom. 6, 21–37. https://doi.org/10.1016/0304-4076(77)90052-5

Ambrose, R.F., Winfrey, B.K., 2015. Comparison of stormwater biofiltration systems in Southeast Australia and Southern California. WIREs Water 2, 131–146. https://doi.org/10.1002/wat2.1064

Andersson, E., Barthel, S., Borgström, S., Colding, J., Elmqvist, T., Folke, C., Gren, Å., 2014. Reconnecting cities to the biosphere: Stewardship of green infrastructure and urban ecosystem services. Ambio 43, 445–453. https://doi.org/10.1007/s13280-014-0506-y

Arneth, A., Sitch, S., Pongratz, J., Stocker, B.D., Ciais, P., Poulter, B., Bayer, A.D., Bondeau, A., Calle, L., Chini, L.P., Gasser, T., Fader, M., Friedlingstein, P., Kato, E., Li, W., Lindeskog, M., Nabel, J.E.M.S., Pugh, T.A.M., Robertson, E., Viovy, N., Yue, C., Zaehle, S., 2017. Historical carbon dioxide emissions caused by land-use changes are possibly larger than assumed. Nat. Geosci. 10, 79–84. https://doi.org/10.1038/ngeo2882

Ball, D.F., 1964. Loss-on-Ignition As an Estimate of Organic Matter and Organic Carbon in Non-Calcareous Soils. J. Soil Sci. 15, 84–92. https://doi.org/10.1111/j.1365-2389.1964.tb00247.x

Baly, E.C.C., 1935. The kinetics of photosynthesis. Proc. R. Soc. B Biol. Sci. 117, 218–239.

Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: Root traits as drivers of ecosystem processes. Trends Ecol. Evol. 29, 692–699. https://doi.org/10.1016/j.tree.2014.10.006

Ben-Noah, I., Friedman, S.P., 2018. Review and evaluation of root respiration and of natural and agricultural processes of soil aeration. Vadose Zo. J. 17. https://doi.org/10.2136/vzj2017.06.0119

Blackman, F.F., 1905. Optima and limiting factors. Ann. Bot. 19, 281–295.

Blouin, M., 2018. Chemical communication: An evidence for co-evolution between plants and soil organisms. Appl. Soil Ecol. 123, 409–415. https://doi.org/10.1016/j.apsoil.2017.10.028

Bousquet, P., Ciais, P., Miller, J.B., Dlugokencky, E.J., Hauglustaine, D.A., Prigent, C., Van Der Werf, G.R., Peylin, P., Brunke, E.G., Carouge, C., Langenfelds, R.L., Lathière, J., Papa, F., Ramonet, M., Schmidt, M., Steele, L.P., Tyler, S.C., White, J., 2006. Contribution of anthropogenic and natural sources to atmospheric methane variability. Nature 443, 439–443. https://doi.org/10.1038/nature05132

Burnham, K. P., and D. R. Anderson. 2004. Multimodel Inference: Understanding AIC and BIC in Model Selection. Sociological Methods & Research 33:261–304.

California Department of Food & Agriculture, 2009. Water supply and demand. URL https://www.cdfa.ca.gov/agvision/docs/Water_Supply_and_Demand.pdf (accessed 11.20.19).

California Department of Water Resources, 2010. Save Our Water Rebates [WWW Document]. URL http://www.saveourwaterrebates.com/turf-replacement-rebates.html (accessed 10.28.19).

California Regional Water Quality Control Board San Diego Region, 2014. National pollutant discharge elimination system permit and waste discharge requirements for discharges from the municipal separate storm sewer systems draining the watersheds within the San Diego region. USA.

Cameron, R.W.F., Blanuša, T., Taylor, J.E., Salisbury, A., Halstead, A.J., Henricot, B., Thompson, K., 2012. The domestic garden - Its contribution to urban green infrastructure. Urban For. Urban Green. 11, 129–137. https://doi.org/10.1016/j.ufug.2012.01.002

Carvalhais, N., Forkel, M., Khomik, M., Bellarby, J., Jung, M., Migliavacca, M., Mu, M., Saatchi, S., Santoro, M., Thurner, M., Weber, U., Ahrens, B., Beer, C., Cescatti, A., Randerson, J.T., Reichstein, M., 2014. Global covariation of carbon turnover times with

climate in terrestrial ecosystems. Nature 514, 213–217. https://doi.org/10.1038/nature13731

Carey, J.C., Tang, J., Templer, P.H., Kroeger, K.D., Crowther, T.W., Burton, A.J., Dukes, J.S., Emmett, B., Frey, S.D., Heskel, M.A., Jiang, L., Machmuller, M.B., Mohan, J., Panetta, A.M., Reich, P.B., Reinschj, S., Wang, X., Allison, S.D., Bamminger, C., Bridgham, S., Collins, S.L., De Dato, G., Eddy, W.C., Enquist, B.J., Estiarte, M., Harte, J., Henderson, A., Johnson, B.R., Larsen, K.S., Luo, Y., Marhan, S., Melillo, J.M., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E., Reinmann, A.B., Reynolds, L.L., Schmidt, I.K., Shaver, G.R., Strong, A.L., Suseela, V., Tietema, A., 2016. Temperature response of soil respiration largely unaltered with experimental warming. Proc. Natl. Acad. Sci. U. S. A. 113, 13797–13802. https://doi.org/10.1073/pnas.1605365113

Chalker-Scott, L., 2007. Impact of mulches on landscape plants and the environment - A review. J. Environ. Hortic. 25, 239–249.

Charnes, A., Cooper, W., Rhodes, E., 1978. Measuring the efficiency of decision making units. Eur. J. Oper. Res. 2, 429–444. https://doi.org/10.1016/0377-2217(78)90138-8

Chen, B., Adimo, O.A., Bao, Z., 2009. Assessment of aesthetic quality and multiple functions of urban green space from the users' perspective: The case of Hangzhou Flower Garden, China. Landsc. Urban Plan. 93, 76–82. https://doi.org/10.1016/j.landurbplan.2009.06.001

Chen, W.Y., 2015. The role of urban green infrastructure in offsetting carbon emissions in 35 major Chinese cities: A nationwide estimate. Cities 44, 112–120. https://doi.org/10.1016/j.cities.2015.01.005

Churkina, G., 2016. The role of urbanization in the global carbon cycle. Front. Ecol. Evol. 3, 144. https://doi.org/10.3389/fevo.2015.00144.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173. https://doi.org/10.1038/nature04514

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecol. Lett. 11, 516–531. https://doi.org/10.1111/j.1461-0248.2008.01164.x

Delphin, S., Escobedo, F.J., Abd-Elrahman, A., Cropper, W.P., 2016. Urbanization as a land use change driver of forest ecosystem services. Land use policy 54, 188–199. https://doi.org/10.1016/j.landusepol.2016.02.006

Demuzere, M., Orru, K., Heidrich, O., Olazabal, E., Geneletti, D., Orru, H., Bhave, A.G., Mittal, N., Feliu, E., Faehnle, M., 2014. Mitigating and adapting to climate change: Multi-functional and multi-scale assessment of green urban infrastructure. J. Environ. Manage. 146, 107–115. https://doi.org/10.1016/j.jenvman.2014.07.025

Diffenbaugh, N.S., Swain, D.L., Touma, D., 2015. Anthropogenic warming has increased drought risk in California. PNAS 112, 3931–3936. https://doi.org/10.1073/pnas.1422385112

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W.W., Scheu, S., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91, 485–496.

Evans, J. R. 2013. Improving photosynthesis. Plant Physiology 162:1780–1793.

Ferreira, I.E.P., Zocchi, S.S., Baron, D., 2017. Reconciling the Mitscherlich's law of diminishing returns with Liebig's law of the minimum. Some results on crop modeling. Math. Biosci. 293, 29–37. https://doi.org/10.1016/j.mbs.2017.08.008

Franklin, J., Serra-Diaz, J.M., Syphard, A.D., Regan, H.M., 2016. Global change and terrestrial plant community dynamics. PNAS 113, 3725–3734. https://doi.org/10.1073/pnas.1519911113

Ge, B., Mehring, A.S., Levin, L.A., 2019. Urbanization alters belowground invertebrate community structure in semi-arid regions: A comparison of lawns, biofilters and sage scrub. Landsc. Urban Plan. 192, 103664. https://doi.org/10.1016/j.landurbplan.2019.103664

Gray, J.T., 1982. Community structure and productivity in ceanothus chaparral and coastal sage scrub of southern California. Ecol. Monogr. 52, 415–434.

Hansen, K., Howitt, R., Williams, J., 2008. Valuing risk: Options in California water markets. Am. J. Agric. Econ. 90, 1336–1342. https://doi.org/10.1111/j.1467-8276.2008.01227.x

Hatt, B.E., Fletcher, T.D., Deletic, A., 2009. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. J. Hydrol. 365, 310–321. https://doi.org/10.1016/j.jhydrol.2008.12.001

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. J. Paleolimnol. 25, 101–110. https://doi.org/10.1023/A:1008119611481

Intergovernmental Planel on Climate Change, 2014. Climate Change 2014 Synthesis Report. Geneva, Switzerland. https://doi.org/10.1017/CBO9781107415324

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3, 315–322. https://doi.org/10.1038/ngeo844

Jassby, A.D., Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21, 540–547. https://doi.org/10.4319/lo.1976.21.4.0540

Jim, C.Y., Chen, W.Y., 2008. Assessing the ecosystem service of air pollutant removal by urban trees in Guangzhou (China). J. Environ. Manage. 88, 665–676. https://doi.org/10.1016/j.jenvman.2007.03.035

Jo, H.K., McPherson, E.G., 1995. Carbon storage and flux in urban residential greenspace. J. Environ. Manage. 45, 109–133. https://doi.org/10.1006/jema.1995.0062

Kayranli, B., M. Scholz, A. Mustafa, and Å. Hedmark. 2010. Carbon storage and fluxes within freshwater wetlands: A critical review. Wetlands 30:111–124.

Laanbroek, H.J., 2010. Methane emission from natural wetlands: Interplay between emergent macrophytes and soil microbial processes. A mini-review. Ann. Bot. 105, 141–153. https://doi.org/10.1093/aob/mcp201

Lerman, S.B., Contosta, A.R., 2019. Lawn mowing frequency and its effects on biogenic and anthropogenic carbon dioxide emissions. Landsc. Urban Plan. 182, 114–123. https://doi.org/10.1016/j.landurbplan.2018.10.016

Levin, L.A., Mehring, A.S., 2015. Optimization of bioretention systems through application of ecological theory. WIREs Water 2, 259–270. https://doi.org/10.1002/wat2.1072

Li, X., Guo, D., Zhang, C., Niu, D., Fu, H., Wan, C., 2018. Contribution of root respiration to total soil respiration in a semi-arid grassland on the Loess Plateau, China. Sci. Total Environ. 627, 1209–1217. https://doi.org/10.1016/j.scitotenv.2018.01.313

Liu, J.S., Lu, L.Y.Y., Lu, W.M., Lin, B.J.Y., 2013. A survey of DEA applications. Omega 41, 893–902. https://doi.org/10.1016/j.omega.2012.11.004

Michaletz, S.T., Kerkhoff, A.J., Enquist, B.J., 2018. Drivers of terrestrial plant production across broad geographical gradients. Glob. Ecol. Biogeogr. 27, 166–174. https://doi.org/10.1111/geb.12685.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington DC. https://doi.org/10.1196/annals.1439.003

Montalvo, A.M., Koehler, C.E., Riordan, E.C., Beyres, J.L., 2017. Plant profile for Artemisia californica.

Moreno-Mateos, D., M. E. Power, F. A. Comín, and R. Yockteng. 2012. Structural and functional loss in restored wetland ecosystems. PLoS Biology 10.

Morse, N.R., Lieberman, M., Todd Walter, M., 2019. Comparing Greenhouse Gas Fluxes from Passive Urban Stormwater Management to Conventional Wastewater Treatment. J. Sustain. Water Built Environ. 5, 04018017. https://doi.org/10.1061/JSWBAY.0000874

Nowak, D.J., Greenfield, E.J., Hoehn, R.E., Lapoint, E., 2013. Carbon storage and sequestration by trees in urban and community areas of the United States. Environ. Pollut. 178, 229–236. https://doi.org/http://dx.doi.org/10.1016/j.envpol.2013.03.019

Payne, E.G.I., Fletcher, T.D., Russell, D.G., Grace, M.R., Cavagnaro, T.R., Evrard, V., Deletic, A., Hatt, B.E., Cook, P.L.M., 2014. Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofiltration systems. PLoS One 9, e90890. https://doi.org/10.1371/journal.pone.0090890

Payne, E., Hatt, B., Deletic, A., Dobbie, M., McCarthy, D., Charasena, G., 2015. Adoption Guidelines for Stormwater Biofiltration Systems - Summary Report. Melbourne.

Platt, T., Denman, K.L., Jassby, A.D., 1975. The mathematical representation and prediction of phytoplankton productivity, Fisheries and Marine Service Technical Report.

Pouyat, R. V., Yesilonis, I.D., Golubiewski, N.E., 2009. A comparison of soil organic carbon stocks between residential turf grass and native soil. Urban Ecosyst. 12, 45–62. https://doi.org/10.1007/s11252-008-0059-6

Raciti, S. M., P. M. Groffman, J. C. Jenkins, R. V. Pouyat, T. J. Fahey, S. T. A. Pickett, and M. L. Cadenasso. 2011. Accumulation of Carbon and Nitrogen in Residential Soils with Different Land-Use Histories. Ecosystems 14:287–297.

Scharenbroch, B., S. Day, T. L. E. Trammell, and R. V. Pouyat. 2017. Urban soil carbon storage. Pages 137–154 in R. Lal and B. A. Stewart, editors. Urban Soils. CRC Press.

Sierra, C.A., Trumbore, S.E., Davidson, E.A., Vicca, S., Janssens, I., 2015. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. J. Adv. Model. Earth Syst. 7, 335–356. https://doi.org/10.1002/2014MS000358

Shchepeleva, A.S., Vasenev, V.I., Mazirov, I.M., Vasenev, I.I., Prokhorov, I.S., Gosse, D.D., 2017. Changes of soil organic carbon stocks and CO 2 emissions at the early stages of urban turf grasses' development. Urban Ecosyst. 20, 309–321. https://doi.org/10.1007/s11252-016-0594-5

Smith, E.L., 1936. Photosynthesis in relation to light and carbon dioxide. PNAS 22, 504–511.

Steele, J.H., 1962. Environmental control of photosynthesis in the sea. Limnol. Oceanogr. 7, 137–150.

Townsend-Small, A., Czimczik, C.I., 2010. Carbon sequestration and greenhouse gas emissions in urban turf. Geophys. Res. Lett. 37, L02707. https://doi.org/10.1029/2009GL041675

U.S. Environmental Protection Agency, 2019. What is green infrastructure? [WWW Document]. URL https://www.epa.gov/green-infrastructure/what-green-infrastructure (accessed 10.28.19).

United Nations Habitat, 2011. Cities and climate change: Global report on human settlements 2011.

United Nations, 2019. Goal 13: Take urgent action to combat climate change and its impacts [WWW Document]. URL https://www.un.org/sustainabledevelopment/climate-change/ (accessed 10.31.19).

van Doren, D., Driessen, P.P.J., Runhaar, H., Giezen, M., 2018. Scaling-up low-carbon urban initiatives: Towards a better understanding. Urban Stud. 55, 175–194. https://doi.org/10.1177/0042098016640456

Velasco, E., Roth, M., Norford, L., Molina, L.T., 2016. Does urban vegetation enhance carbon sequestration? Landsc. Urban Plan. 148, 99–107. https://doi.org/10.1016/j.landurbplan.2015.12.003

Wahbi S., et al., 2015. Increasing the Role of Mycorrhizal Symbiosis in Plant-Plant Facilitation Process to Improve the Productivity and Sustainability of Mediterranean Agrosystems. In: Arora N. (eds) Plant Microbes Symbiosis: Applied Facets. Springer, New Delhi

Wang, Y., de Groot, R., Bakker, F., Wörtche, H., Leemans, R., 2017a. Thermal comfort in urban green spaces: a survey on a Dutch university campus. Int. J. Biometeorol. 61, 87–101. https://doi.org/10.1007/s00484-016-1193-0.

Wang, C., Ma, Y., Trogisch, S., Huang, Y., Geng, Y., Scherer-Lorenzen, M., He, J.S., 2017b. Soil respiration is driven by fine root biomass along a forest chronosequence in subtropical China. J. Plant Ecol. 10, 36–46. https://doi.org/10.1093/jpe/rtw044

Wang, H.M., Saigusa, N., Zu, Y.G., Wang, W.J., Yamamoto, S., Kondo, H., 2008. Carbon fluxes and their response to environmental variables in a Dahurian larch forest ecosystem in northeast China. J. For. Res. 19, 1–10. https://doi.org/10.1007/s11676-008-0001-z

Wheeler, M. M., M. M. Dipman, T. A. Adams, A. V. Ruina, C. R. Robins, and W. M. Meyer. 2016. Carbon and nitrogen storage in California sage scrub and non-native grassland habitats. Journal of Arid Environments 129:119–125.

Wilson, T.S., Sleeter, B.M., Richard Cameron, D., 2016. Future land-use related water demand in California. Environ. Res. Lett. 11, 054018. https://doi.org/10.1088/1748-9326/11/5/054018

Chapter 7: Conclusions

Jennifer T. Le

Ecosystems not only provide targeted benefits, such as commercial products and designed mechanisms, but a wealth of ecosystem services that contribute to human well-being. They present a tradeoff that environmental decision-makers must internalize and consider, especially as demand for natural resources continues to increase. This dissertation investigated ecosystem services in a multitude of systems and, in doing so, presents three main conclusions: (1) deep-sea habitats and natural stormwater treatment systems (NTS) provide a diversity of ecosystem services that manifest through distinct pathways; (2) existing scientific tools can be leveraged to characterize, quantify, and economically value ecosystem services; and (3) an interdisciplinary approach can facilitate relevancy of scientific results to environmental decision-makers.

Diverse ecosystem services in different systems

The focus on two types of systems presented an opportunity to compare and contrast the study ecosystem services and application of results. At first glance, deep-sea habitats and NTS have nothing in common: the former are marine, remote, and experience relatively little human impact, whereas the latter are terrestrial, urban, and human-designed. However, a closer look at these systems reveals some similarities. While some deep-sea ecosystem services operate on relatively large spatial scales and long time scales (Chapter 2), those found on methane seeps (Chapter 4) can be localized on similar spatial scales as NTS (Chapter 5). Methane seeps and

NTS both provide climate-regulating services related to carbon, but the processes by which they do so are different: at methane seeps, carbonates precipitate as a result of microbial activity (Marlow et al., 2014), and in NTS, photosynthesis converts atmospheric carbon dioxide into organic matter. Although the final services are similar, further investigation of their supporting structures and functions revealed mechanisms unique to each system. This may mean that consideration of only final ecosystem services is insufficient to establish offsets and other mitigation options.

The state of environmental rules, regulations, and policies also vary among the two systems. Deep-sea management is currently being developed for some industries which presents an opportunity to incorporate ecosystem services before commercial exploitation. Including indicators of ecosystem services now could ensure extensive baseline data to which to compare impacts against in the future. In contrast, there are extensive guidelines on design and installation of NTS. However, they often focus on targeted water services but consideration of other ecosystem services could provide alternative design and management options. While the study of ecosystem services in the deep sea and NTS may seem incongruent, there was a lot to be learned in doing so.

Leveraging existing scientific tools

This dissertation assessed multiple scientific tools and approaches to studying ecosystem services, including molecular techniques, *in situ* sensors, and methods from several academic disciplines. Molecular techniques can be applied to both systems but present distinct challenges in each. Molecular data can be difficult to interpret in deep-sea habitats that have relatively small sequence databases. In contrast, urban soil microbes have been better catalogued (e.g. Joyner et

al., 2019), which may facilitate the use of molecular techniques to characterize ecosystem services in NTS. In part, this may be because sampling in the deep sea often necessitates ship time and specialized instrumentation (e.g. ROVs), whereas sampling in NTS can be done more easily (e.g. portable field equipment). *In situ* sensors are effective at measuring chemical concentrations and fluxes, likely in any system, but there are specific marine challenges to consider such as corrosion and battery life. In some cases, data (e.g. repositories of deep-sea imagery) and tools (e.g. data envelopment analysis) already exist but an ecosystem services perspective has yet to be applied. This dissertation employed novel approaches to the study of ecosystem services and revealed the potential for leveraging existing resources in creative ways.

An interdisciplinary approach

The study of ecosystem services is inherently interdisciplinary, tying together the disciplines of natural and social sciences. Chapter 2 explicitly highlights where ecosystem services could be utilized in deep-seabed mining regulations, such as designation of protected areas. This necessitates indicators of ecosystem services in baseline assessments and monitoring programs for identification of high-value areas. Quantification of ecosystem services could then allow for economic valuation to facilitate payment for environmental damages. The concept of ecosystem services seamlessly weaves together the physical, chemical, and biological sciences necessary to understand ecosystem services with economics and policy to make natural processes relevant outside of academia.

Approaches from other academic disciplines can produce interesting results with a unique perspective. Chapter 3, which discusses available approaches for biodiversity assessment and monitoring, incorporates an economic cost-effectiveness analysis that is likely of interest to

decision-makers who will be choosing how the deep-seabed mining industry conducts their data collection. In NTS, there is a unique opportunity to manipulate the system because they are human-built. Although NTS were not found to be carbon sinks (Chapter 6), an operations research approach identified potential maintenance and management adjustments (e.g. amount of irrigation) to reduce carbon emissions of NTS and other urban landscapes. There may be tradeoffs in doing so, but the results provide further options for urban planners and managers.

Next steps and closing remarks

This body of work represents an exploration of ecosystem services in systems where they have not been fully utilized. The next step seems obvious but is likely more complicated than imagined as life is wont to be: quantification of ecosystem services. Recommendations include (1) to develop practical ecosystem services indicators, and (2) to ensure accountability for ecosystem services. Many people, including the author of this dissertation, pay tribute to ecosystem services but their actual rates and values are unknown in many cases (Guerry et al., 2015). One way to address this is to develop practical indicators that can determine their stocks and flows (Heink et al., 2016). These indicators need to be based on sound scientific methods as well as available and accessible to environmental planners and managers. Validation of ecosystem services necessitates accountability for actions that impact them. While the first step is quantification, it can lead to economic valuation of ecosystem services. That is not to say there are no other values associated with ecosystem services (Martin-Lopez et al., 2014). However, compensation for loss of benefits can help internalize environmental damages into decision-making and yield more optimal results.

This dissertation was inspired by the idea of balance: how can we balance extraction of

natural resources and protection of the environment? This question brought me, and readers of this work, to the concept of ecosystem services. Although there are some people who are uncomfortable with "putting a value on nature," ecosystem services are simply a shift in perspective when considering natural processes, one that is relevant to environmental issues and challenges we face now. To conclude, I hope this dissertation has demonstrated that the concept has the potential to, not only advance our understanding of the natural world, but also advance our ability to benefit from and protect it.

References

Guerry, A. D., S. Polasky, J. Lubchenco, R. Chaplin-Kramer, G. C. Daily, R. Griffin, M. Ruckelshaus, I. J. Bateman, A. Duraiappah, T. Elmqvist, M. W. Feldman, C. Folke, J. Hoekstra, P. M. Kareiva, B. L. Keeler, S. Li, E. McKenzie, Z. Ouyang, B. Reyers, T. H. Ricketts, J. Rockström, H. Tallis, and B. Vira. 2015. Natural capital and ecosystem services informing decisions: From promise to practice. Proceedings of the National Academy of Sciences of the United States of America 112:7348–7355.

Heink, U., J. Hauck, K. Jax, and U. Sukopp. 2016. Requirements for the selection of ecosystem service indicators - The case of MAES indicators. Ecological Indicators 61:18–26.

Joyner, J. L., J. Kerwin, M. Deeb, G. Lozefski, B. Prithiviraj, A. Paltseva, J. McLaughlin, P. Groffman, Z. Cheng, and T. R. Muth. 2019. Green infrastructure design influences communities of urban soil bacteria. Frontiers in Microbiology 10.

Marlow, J. J., J. A. Steele, W. Ziebis, A. R. Thurber, L. A. Levin, and V. J. Orphan. 2014. Carbonate-hosted methanotrophy represents an unrecognized methane sink in the deep sea. Nature Communications 5.

Martín-López, B., E. Gómez-Baggethun, M. García-Llorente, and C. Montes. 2014. Trade-offs across value-domains in ecosystem services assessment. Ecological Indicators 37:220–228.

Smith, K. L., H. A. Ruhl, B. J. Bett, D. S. M. Billett, R. S. Lampitt, and R. S. Kaufmann. 2009. Climate, carbon cycling, and deep-ocean ecosystems. Proceedings of the National Academy of Sciences 106:19211–19218.